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PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XVIII. THE RELATIONSHIP BETWEEN THE BEAKER TEST AND FACIAL-ECZEMA TOXICITY

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(*Received for publication, 21 July 1959*)

Summary

The relationship between the beaker test and facial-eczema toxicity is examined, using all results obtained in 1957 and 1958. If samples stored in sacks for several months before feeding in 1957 are included, the beaker test does not appear to be a reliable indicator of toxicity. This is attributed to loss of toxicity during storage. For samples collected late in the autumn of 1957, a positive beaker test did not indicate toxicity. This is ascribed to retention of the beaker-test substance in the pasture after toxicity had ceased. For samples collected in 1957 during the toxic period and fed within 14 weeks, there is a close association between the beaker test and toxicity detected by guinea pig feeding. Samples obtained during 1958 show a less marked relationship between the two tests.

The use and limitations of the beaker test and factors which may influence its reliability are discussed.

INTRODUCTION

Perrin (1959) described a chemical procedure (the "beaker test") for indicating facial-eczema toxicity in pasture and presented results showing a relationship between this test and toxicity assessed by guinea pig feeding. Those results included 184 samples collected during the period when facial eczema was likely to occur in 1957, but one third of these were non-toxic samples from a district where no outbreak occurred. In the present paper this relationship is further examined, using the results from all the samples collected during February to April 1957, including those considered by Perrin (1959). Particular attention is given to samples from the Gisborne district where liver damage and clinical facial eczema occurred on some of the sampling areas. Certain factors which became apparent during the guinea pig feeding trials are considered in interpretation of the results.

The relationship between the two tests applied to a smaller group of samples from the Waikato district in 1958 is also reported.

Methods

The procedures for the beaker test and for guinea pig feeding for the 1957 samples were those described by Perrin (1959). The reference in that paper to use of standards in grading the 1957 beaker tests is, however, not strictly accurate. Standards were prepared from typical beaker-test-positive grasses, but these deteriorated through being left uncovered and were not used routinely. The phenolphthalein standard described by Perrin (1959) was not introduced until most of the beaker testing had been completed, and was also not used because the phenolphthalein deposit does not closely simulate the beaker test. Grading of the tests was, therefore, entirely subjective, and although agreement was usual between gradings by two independent observers, the assessment appears to have changed with experience.* Thus a trace of deposit which was classed as negative in early tests, was later graded as positive.

For the 1958 samples the procedure described by Sandos *et al.* (1959) was used, with comparison against standards graded 1 to 3.

Results and Discussion

Samples from Waikato District, 1957

Pasture was collected every second day from 11 areas within 30 miles of Hamilton. Three areas contained pennyroyal which gives a false beaker test by the procedure used (Perrin 1959; White 1958) so that samples from these areas were not considered. From the remaining eight areas, 165 samples were fed to guinea pigs, but only two, which were consecutive cuts from one area, proved toxic. Both these samples gave positive beaker tests. Four non-toxic samples also gave positive beaker tests, and it is of interest that two of these were the cuts immediately preceding and following the two toxic samples with positive beaker tests.

Samples from Gisborne District, 1957

Samples were mown daily from one area over the period 31 January to 18 April, and at frequent intervals from other fields on the same farm. On four other areas pasture was collected every second day over most of this period. Clinical facial eczema occurred in lambs grazing the area sampled daily, and in lambs on fields adjoining some of the other cutting areas, and toxic grass was obtained from all collection areas.

A total of 228 samples were tested by feeding to one guinea pig and by the beaker test. A comparison of results by the two methods is shown below.

	G.P. +	G.P. —
Beaker test +	65	69
Beaker test —	19	75

This comparison does not indicate a close relationship between the beaker test and toxicity to the guinea pig.

* One of us (J.S.) carried out or supervised the tests made during 1957.

The beaker tests on all these samples were completed within a few weeks, but owing to the large number of samples and the need to feed samples for five weeks, testing for toxicity was spread over 6 months. During this time several factors which might influence interpretation of the results became apparent.

Variation in Guinea Pig Susceptibility

Recent work (unpublished) has shown that in a group of guinea pigs fed on one sample a wide range of response can occur. It is probable that the system of feeding to only one guinea pig, adopted primarily to select, from a large number of samples, those sufficiently toxic for chemical extraction, failed to detect some toxic samples, especially in the lower toxicity grades. No allowance is made in this paper for error arising from this source, which would, in any case, be common to the various groups of samples considered.

Loss of Toxicity During Storage

Before feeding, samples were stored in sacks in a dry place, a system which had appeared satisfactory for about 12 months in a previous year. When feeding had been in progress about 14 weeks, negative results were obtained in repeat tests on samples which had proved highly toxic when first fed, and thereafter about 50% of repeat feedings gave normal livers or less damage than previously recorded. Not all these differences are due to loss of toxicity during storage, and some may be accounted for by variation in guinea pig susceptibility, since a few samples which were negative at the second test have produced lesions in subsequent feeding. However, the following data suggest that considerable loss of toxicity has occurred: (a) When repeat guinea pig tests were carried out on 38 samples originally graded mild to moderately severe, and which could, therefore, either increase or decrease in toxicity on subsequent feedings, 6 samples retained the same grading, and 4 increased in grading, whereas 28 decreased. (b) When similar repeat tests were run on 28 samples negative at the first tests, although samples mown from the same field two days before and two days after them were toxic, none of these were positive at the second feeding.

Persistence of Positive Beaker Test After Toxicity Ceased

Positive beaker tests were recorded on several areas until early in April, several weeks after the toxic period, as judged from field experience, had passed. It appeared likely, therefore, that the beaker test substance, which is considered to be more stable than the hepatotoxin, may continue to be produced or is retained in herbage as collected by mowers after the toxin itself had disappeared.

In view of these factors the comparison between the beaker test and guinea pig toxicity was re-examined along the following lines:—

By Period between Cutting and Completion of Feeding. The samples were divided into two groups, those fed within 14 weeks, and those fed 14 weeks or more after cutting. Fourteen weeks was chosen initially because this was the interval between collection and testing for

the first repeat feedings which proved negative. In fact, it corresponds to a natural division because only a small proportion (8%) of the samples were fed at intervals between 12 and 15 weeks, and because approximately half the samples fall in each group.

By Date of Cutting. For this purpose the samples were divided into 4 groups. The March period was terminated at 25 March because evidence from serial killing of lambs on the main cutting area, by the procedure described by Simpson *et al.* (1957) indicated that toxicity to grazing lambs had ceased by this date. This period was further subdivided because of the large number of samples collected over this time, when pasture conditions were changing rapidly.

The results of classification of the samples into these groups are given in Table 1.

TABLE 1. COMPARISON BETWEEN BEAKER TEST AND GUINEA PIG TOXICITY IN DIFFERENT PERIODS

Cutting Period		Interval, Cutting to Feeding				Totals	
		Within 14 wks		Over 14 wks			
		G.P. +	G.P. —	G.P. +	G.P. —	G.P. +	G.P. —
1st Period	B.T. +	21	1	3	6	24	7
Jan 31-Feb 28	B.T. —	14	20	1	17	15	37
2nd Period	B.T. +	17	1	2	8	19	9
Mar 1-12	B.T. —	3	1	0	5	3	6
3rd Period	B.T. +	15	2	6	24	21	26
Mar 13-25	B.T. —	0	4	1	4	1	8
Total 1st, 2nd, 3rd	B.T. +	53	4	11	38	64	42
Jan 31-Mar 25	B.T. —	17	25	2	26	19	51
4th Period	B.T. +	0	13	1	14	1	27
Mar 26-Apl 18	B.T. —	0	4	0	20	0	24
Totals	B.T. +	53	17	12	52	65	69
	B.T. —	17	29	2	46	19	75

Attention is drawn to the following features in Table 1:

(1) It is apparent that the proportion of samples that gave positive beaker tests and also gave liver damage is very high in the group of samples fed within 14 weeks (53 out of 70, or 76%), in comparison with the corresponding proportion in samples fed after 14 weeks or more (12 out of 64 or 19%). This difference between the two groups is found in the first three cutting periods, but not in the fourth, and over these first three periods the difference in the proportions is still more marked—94% compared with 22%.

This influence of interval before feeding on agreement between beaker test and guinea pig test is further indicated by results for 30 samples cut from one area on consecutive days from 19 February. Of

15 fed 14 weeks or more after cutting, 6 which were toxic were cut on days, 1, 5, 21, 26, 28, 29; and 9 which were not toxic were cut on days 3, 7, 9, 10, 13, 15, 19, 20, 27. The remaining 15, cut on the intervening days, were all fed within 14 weeks, and were all toxic.

Such a difference between these two groups of samples is consistent with loss of toxicity in those for which feeding was delayed, and no other explanation can be offered.

(2) Among those cut up to 25 March, 64 out of 106 that gave positive beaker tests were positive by the guinea pig test also (i.e. 62%), whereas of those cut after 25 March, the corresponding ratio is 1 out of 28 (4%). This difference is more marked when only samples fed within 14 weeks are considered (94% compared with 0%). The progressive decrease in this ratio during the first three periods is accounted for by the increase in the proportion of late-fed samples in the later periods.

This decrease in the proportion of positive beaker tests associated with toxicity towards the end of the cutting period, and at a time when field evidence indicated that the pasture was no longer toxic to grazing sheep, suggests that the white material responsible for the test remains, or continues to be produced, in herbage collected by mowers after the hepatotoxin has disappeared. The beaker test substance, unlike the hepatotoxin, is chemically stable (White 1958). Perrin (1959) recorded, and subsequent experiments have confirmed, that dry stalks and other debris in some samples gave a positive beaker test. Such material derived from earlier toxic growth could continue to be picked up by mowers together with much more recent non-toxic green leaf.

This persistence of the beaker test beyond the toxic period is not so apparent from the analysis of the results presented by Perrin (1959) since his paper included only eight samples cut after 25 March. Of those eight, five gave positive beaker tests but none was toxic. (The two samples recorded in Fig. 1 of Perrin's paper as toxic after this date did not show facial-eczema lesions on histological examination.)

(3) A further feature of Table 1 is the high proportion of samples in the first period which were negative by beaker test but proved toxic on feeding. When these samples were retested later all except two gave a positive beaker test. This anomaly appears to have arisen through the subjective nature of the assessment of the beaker test grading referred to in the Introduction, and there is little doubt that the later assessments are correct. However, because very few of the samples negative by both beaker test and guinea pig test were available for similar re-testing, only the initial test has been used in the analysis for Table 1. Of five samples negative by both tests which were available, none was positive on re-examination.

In considering the foregoing analysis it should be pointed out that trends similar to those seen in Table 1 are obtained if the samples are grouped according to the month in which they were fed, instead of by interval between cutting and feeding. The proportions of samples positive by beaker test which were also positive by guinea pig feeding were, in March-April-May, 96%; in June 50%; and in July-August

18%. There is no evidence to suggest that there was any undetermined change in sampling, preparation of samples, feeding technique, etc., which might have given erroneous results during the latter half of the feeding period. On the other hand, because samples which were cut early tended to be fed without undue delay, division of the samples by month of feeding and by interval before feeding produce very similar groups. Thus the group fed March-May contained 80% of those fed within 14 weeks, and none cut after 25 March; whereas the July-August group included only 10% of the "within 14 weeks" samples and the majority of those cut after 25 March.

Grading of Beaker Test in Relation to Guinea Pig Toxicity

In the previous paper (Perrin 1959) an attempt was made to relate the grade of the beaker test to the toxicity of the sample. Considering the possible errors, already referred to, in assessing both beaker test and toxicity, a close correlation is not to be expected. However, among the 99 Gisborne samples cut before 26 March and fed within 14 weeks, 39 out of 48 graded 2 or 3 gave liver damage in the classes "moderately severe" or "severe", whereas only 12 out of 51 graded 0 or 1 fell in these classes.

Application of Beaker Test

The procedure used to obtain the 1957 results presented in this paper would necessitate 36 to 48 hours delay between sampling a field and returning to collect a quantity of grass if the test is positive. The procedure now in use (Sandos *et al.* 1959) requires only about 4–6 hours, so that an area can be sampled, tested, and mown within one day. It is valid, therefore, to assess the usefulness of the beaker test by direct comparison with toxicity.

In view of the foregoing discussion, only those samples fed within 14 weeks of cutting are considered for this purpose. If the beaker test had been used as a guide for cutting these samples, 70 large-scale cuts would have been made instead of 116 and 55 of these would have been toxic, but 17 toxic samples would have been missed. In practice, for the collection of grass sufficiently toxic for chemical extraction, it may be more satisfactory to cut only areas giving a beaker test of grade 2 or higher. On this basis the 116 collections would have been reduced to 57, of which 39 gave moderate or severe liver damage, and 11 samples of this grading would have been missed.

The evidence that the beaker test persists beyond the toxic period indicates that the test is of little value late in the season. This is illustrated by the fact that 11 of the 15 non-toxic samples which would have been collected by using the beaker test as a guide were among those cut after 25 March. The test is, in fact, likely to be reliable only during the first period of toxicity in any season. This considerably limits its application, in particular ruling out any use to determine when pastures become safe for grazing. Its main use at present is likely to be the choice of areas, early in the season, for systematic grass collection or other field experiments.

Although the proportion of toxic samples which would have been missed through reliance on the beaker test (17 out of 72, or 24%) is very high, 12 of these were of low grade toxicity, and in any case 15 can be accounted for by incorrect assessment in the early stages of the testing. As an illustration of the value of negative tests in preventing unnecessary collection, the data for the Waikato samples are quoted. Here only 6 instead of 165 would have been cut, yielding two toxic samples and missing none.

Samples Collected During 1958

Gisborne District. Samples were collected regularly during autumn 1958 from 5 areas in this district, but no positive beaker tests were recorded. As no liver damage occurred in lambs grazing experimental plots and the district generally was free from eczema, these samples were not fed to guinea pigs.

Waikato District. There were very few outbreaks of clinical eczema in this district in 1958, although liver lesions were seen in many livers of lambs at meat works. The results presented below are for 138 samples collected from eight areas. In this series there is no evidence of loss of toxicity before feeding, the grass being packed into tins immediately after drying and stored at —20°c. Each sample was fed to two or more guinea pigs, but unlike the 1957 samples, those of 1958 were not ground before feeding.

	G.P. +	G.P. —
B.T. +	27	29
B.T. —	6	76

Unfortunately regular collection of samples large enough for guinea pig testing ceased on 19 March and, although a number of positive beaker tests were recorded after this date, only three were available for feeding, one of these (3 April) being toxic. Consequently the end of the toxic period cannot be closely defined, and there is no information in this year regarding continuation of beaker tests after toxicity ceased.

The above results confirm an association between a positive beaker test and toxicity, since 50% of the B.T.+ samples were toxic against only 7% of the beaker test negative; but the association is less close than indicated by the analysis of the 1957 results (Table 1).

Subsequent to the feeding of the 1958 samples it has been established that a fungus, *Sporidesmium bakeri* Syd., which is saprophytic upon dead pasture litter, produces both the beaker test substance and a toxic principle causing liver lesions identical with those of facial eczema (Percival and Thornton 1958; Thornton and Percival 1959). This discovery suggests several possible explanations for the difference between the 1957 and 1958 results and for other apparent anomalies in the association between beaker test and toxicity.

(a) The general level of both the beaker tests and toxicity was low in the 1958 material in comparison with pasture from Gisborne in

1957. Of the 29 B.T.+, G.P.— samples in 1958, 22 were Grade 1 samples from areas which produced toxic grass a few days before or after these non-toxic samples were collected. It is possible that some or all of these 22 samples contained the toxin in amounts too low to detect by direct feeding. By feeding ether extracts at high level, Perrin (1959) detected the toxin in some beaker-test-positive samples which were non-toxic when fed direct.

(b) In 1957 the dried grass was ground before feeding, but in 1958 it was fed unground, a procedure considered at that time more satisfactory because the animals selected green material, rejecting most of the dried stalk and debris. However, with samples in which most of the fungus was on the litter, this feeding technique may have failed to detect toxicity, whereas beaker tests made on the ground material were positive.

Apart from these hypotheses involving technique, other factors, on which little information is yet available, may underlie these and other anomalies in the association between the beaker test and the hepatotoxin. The basis of this association is the production of both substances by *Sporidesmium bakeri;* but it is unlikely that this fungus always produces both at the same time or in fixed proportions, and it may not be the only organism which can give rise to a beaker test in mown pasture. Tests on a number of micro-organisms isolated from pasture and grown in pure cultures have already revealed one (*Fusarium avenaceum*) which gives a positive beaker test. The presence of such an organism might account for the 7 non-toxic samples, with beaker test higher than grade 1, which were recorded in 1958, most of these being mown from one area 10 days or more before the first toxic sample was cut.

At present nothing is known of the metabolic or chemical relationships between the two substances or of how much their association depends on their relative stabilities, or on strain, stage of growth, and substrate of the fungus. The beaker-test material has often enough been found in pastures when the toxin is not detectable; it cannot be assumed that the toxin is never present when the beaker test substance is absent.

The results presented in this paper confirm a frequent association between a positive beaker test and toxicity, but indicate that this association is not invariable. The test is in fact primarily an indicator of the presence of the fungus *Sporidesmium* rather than of facial-eczema toxicity. As such it provides a valuable research tool, but its use as a basis of field control measures is not recommended.

Acknowledgements

We are indebted to Miss A. Stevenson and Mr. H. de Langen for carrying out many of the routine beaker tests during 1958, and to Dr P. J. Brooke for providing cultures of *Fusarium* and other organisms.

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EFFECT OF SHEARING TIME ON WOOL

IV. EFFECT ON THE FLEECES

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(*Received for publication, 4 August 1959*)

Summary

A comparison of the effect of shearing before instead of after lambing, made on the two halves of the same flock of ewes, has been made in four successive years on a flock of Romneys and two years on a flock of Corriedales. In both flocks the pre-lamb-shorn fleeces were consistently much superior in soundness. Thus eight post-lamb-shorn fleeces were down-graded because of wool faults for every one pre-lamb-shorn fleece. Staples from pre-lamb-shorn fleeces were two to two-and-a-half times as strong as those from post-lamb-shorn fleeces. Time of shearing resulted in no consistent difference in fleece weight or length in either flock.

Introduction

In the previous papers in this series (Story 1955) it was shown that there were two aspects of the effect on the wool of shearing before lambing, firstly the effect on the grading of the fleeces, and secondly the effect on the processing performance of the wool. In this paper, the comparative results of the effect of shearing before or after lambing on the Romney crossbred flock at the Invermay Research Station, Mosgiel, and on a Corriedale flock near Waimate are given. Data are now available for four years on the Romney flock and two years on the Corriedale flock, and the results are so much in favour of pre-lamb shearing that no further observations are being made on sheep of these breeds.

Composition of the Flocks

Romney Ewes

The trials at Invermay were commenced in 1952, and the results of the first shearings in 1953 were reported in Part III of this series (Story 1955). Subsequent shearings were made in 1954, 1955, and 1956. A summary of the earlier data is included here for the sake of completeness.

The original flock was composed of about 300 Romney ewes of mixed ages, half of which were shorn in August before lambing and the other half in November after lambing. At each shearing approximately 30 ewe hoggets were also shorn and added to the flock as replacements. The sheep were all ear-tagged with numbered tags so that they could be individually identified. They were all run together on pastures, with some supplementary winter feed, and all had the same treatment.

Corriedale Ewes

This trial was made possible by the courtesy of Mr. G. Barclay, of Crestlow Station, Waihaorunga, near Waimate, who very kindly put

* Formerly New Zealand Woollen Mills Research Association.

a group of about 200 Corriedale 2-tooth ewes at our disposal in 1954. The ewes were selected by eye appraisal as being similar in wool quality and were divided into two groups at random, which were then distinguished by different-coloured ear-tags (unnumbered). One group was shorn in September and the other in December (January, 1957) but apart from this they all received the same treatment as at Invermay, and were run on hill pastures for most of the year.

SHEARING AND CLASSING PROCEDURE

The procedure was basically the same for the two flocks. The fleeces were weighed immediately after shearing, skirted and re-weighed. Four staples were taken along one side for measurement of length.

The locks and bellies were collected and weighed at the end of shearing, and an average correction was made to the fleece weights. In addition, data were also obtained for the Romney sheep in 1954-6 giving the average weights of crutchings and bellies removed between shearings, which differed for the two groups because of the different time intervals between shearing and crutching. These data were also applied as corrections to the fleece weights to give the total annual greasy wool production from the sheep.

The Romney fleeces were classed for quality (fineness) and graded as "good" or "faulty", details of all faults being recorded. The Corriedale fleeces were classed for quality and graded for style in more detail on the Bareme scale, all faults being recorded.

FLEECE WEIGHTS AND LENGTHS

The interval between successive shearings was not exactly 52 weeks, as there was sometimes a delay due to wet weather, particularly with

TABLE 1. FLEECE WEIGHTS AND LENGTHS*

	Year	Pre-lamb-shorn			Post-lamb-shorn		
		No. of Fleeces	Mean Weight lb	Mean Length in.	No. of Fleeces	Mean Weight lb	Mean Length in.
Romney	1953	153	8.5	6.8	135	9.1	7.2
Romney	1954	150	11.3	7.2	125	11.0	7.1
Romney	1955	152	13.2	7.2	103	12.4	7.1
Romney	1956	133	9.2	5.2	105	9.5	5.2
Mean			10.5	6.6		10.5	6.6
Corriedale	1955	94	8.5	4.8	95	8.5	4.6
Corriedale	1956	80	7.0	3.7	97	7.2	3.5
Mean			7.8	4.3		7.9	4.1

* Corrected to correspond to 52 weeks' growth, and including average weights of crutchings and bellies for the 1954-6 Romney wools.

the post-lamb shearings. In the tabulated results (Table 1) that follow, the wool weights and lengths have been calculated to the equivalent of 52 weeks' growth to facilitate comparison. This correction was made by using the rate of growth data to be presented in Part VI.

The figures show a considerable variation in wool production from year to year. Thus the Romney fleeces were noticeably heavier in 1954 and 1955 than they were in 1953 and 1956. Similarly the Corriedale fleeces were lighter in 1956. The last season had been notable for a dry autumn with lack of feed and also for failure of winter turnip crops, and this is the reason for the low weights in 1956; the pre-lamb-shorn clips were affected more than the post-lamb-shorn as the latter had the benefit of a good early spring.

With reference to the shorter lengths recorded in 1956, these were partly due to the poorer seasonal conditions, but also partly to the measurements being made by a different operator.

STYLE GRADINGS

Faults in the fleeces were classified under two headings, namely "wool" faults which included cotting, tenderness, break, mushy backs, and coloured yolk, and "non-wool" faults, which included dust, moit (vegetable matter), and other acquired faults. Faulty pre-lamb-shorn fleeces usually only had one fault, whereas post-lamb-shorn fleeces commonly had two and sometimes more faults. Such fleeces were classified under the fault considered to be most serious (and were only included once).

The incidence of faults in the various clips is described below.

TABLE 2. FLEECE GRADINGS OF ROMNEY WOOLS

Year	1953		1954		1955		1956	
	A* %	B* %	A %	B %	A %	B %	A %	B %
Wool faults:								
Cotted and tender	—	—	—	28	—	8	—	42
Cotted only	3	5	1	—	1	—	1	16
Tender only	—	18	5	6	1	52	1	22
Mushy	5	—	1	—	1	—	1	—
Coloured yolk		12	3	18	1	1	11	10
Total	8	35	10	52	4	61	14	90
Non-wool faults	19	7	12	1	21	10	31	1
Total faults	27	42	22	53	25	71	45	91

* A = pre-lamb-shorn, B = post-lamb-shorn.

Romney. Here the fleeces were graded only as "good" or "faulty", all faults in the latter category being recorded except in the first year when the system had not been perfected. The percentages of faulty fleeces are given in Table 2.

Corriedale. These finer-wool fleeces were graded in more detail, and the percentages of fleeces containing good-grade BB wool and poor-grade C wool, together with the percentages of the various faults, are given in Table 3.

TABLE 3. FLEECE GRADINGS OF CORRIEDALE WOOLS

Year	1955		1956	
	A* %	B* %	A %	B %
Wool faults—				
Cotted	1	7	—	15
Break or tender	—	54	—	69
Mushy	10	10	3	1
Coloured yolk	2	15	3	5
Total	13	86	6	90
Non-wool faults	27	5	7	—
Total faults	40	91	13	90
Fleeces with BB wool	64	3	90	6
Fleeces with C wool	15	45	1	48

* A = pre-lamb-shorn, B = post-lamb-shorn.

It was noticed that there was a tendency for the pre-lamb-shorn wool to be more contaminated with bits of vegetable matter. These seemed to be mostly pieces of chaff and hay, derived from feeding out to the sheep during the winter, and would be expected to drop out of the wool during carding.

Relation between Fleece Weight and Grading

It has been shown by Henderson (1955) that within a flock there is a relationship between fleece weight and the grade of the wool (other things being equal). Such a relationship was found in the results on the post-lamb-shorn fleeces from Invermay in 1954-6 where on the average the faulty fleeces were 0.8 lb lighter than the good fleeces.

In the pre-lamb-shorn clips on the other hand the faults were usually only dust and moit and so there was little relation between fleece weight and grade. Also in 1953 when the wool faults in the post-lamb-shorn clip were not nearly as severe or as numerous as in later years there was little difference in weight between the good and faulty fleeces.

"Half-shorn Sheep"

When a farmer changes from shearing after lambing to shearing before, he can only compare his wool with what he produced 2 seasons before (in the changeover year he will have only 9 months' wool) or with a neighbour's wool. In both cases differences other than shearing-time are likely to prejudice the comparison, e.g. weather, differences between properties and in sheep management, etc.

A significant comparison between pre- and post-lamb-shorn wools can thus only be made when both lots of wool are shorn from different parts of the same flock of sheep at appropriate times, the sheep otherwise all having the same treatment.

This was carried a step further to eliminate all differences other than shearing-time, by shearing one side only of each of 4 ewes in August 1954 and 1955, the other side being shorn in November. In 1954 the two half-fleeces per sheep differed in length, as they represented 12 and 15 months' growth of wool respectively, while in 1955 they had both had 12 months' growth.

The style-grading of the half-fleeces was exactly in line with the grading of the whole fleeces shorn at the different times. Thus all the August-shorn half-fleeces in both years were sound. In November 1954, however, only one half-fleece appeared sound, two were rather tender and the fourth was tender and considerably cotted. They were a little better in November 1955, two of the half-fleeces appearing sound and the other two tender. Thus while all the wool was sound in August it frequently deteriorated badly in the next 3 months over the lambing period.

Staple Strengths

In the wool trade the soundness of a staple is judged subjectively by flicking it with a finger. This test will detect gross tenderness, but does not distinguish between moderately sound and very sound staples.

An objective test of staple strength has been devised at this Institute (Ross 1959), and has been used to measure the actual strengths of midside samples, taken from the sheep just before shearing, from the Romney and Corriedale ewes in 1955 and 1956. A test-length of 2.5 in. was used for Romney samples and 1.5 in. for Corriedales, in both cases

TABLE 4. Staple Strengths

Wool	Year	Test Length in.	Mean Strength, lb/g	
			Pre-lamb	Post-lamb
Romney	1955	2.5	162	61
Romney	1956	2.5	142	58
Corriedale	1955	1.5	238	113
Corriedale	1956	1.5	228	116

commencing at a point approximately 0.75 in. from the butt end. The mean results are given in pounds per gramme of clean wool, in Table 4, and show that pre-lamb-shorn wool is very much stronger than post-lamb-shorn.

Mid-side samples from the "half-shorn" sheep were also tested (test-length 2.5 in.), giving the results shown in Table 5. These results are in excellent agreement with the style gradings, and show that wool from the same sheep is much stronger when shorn before lambing.

TABLE 5. STAPLE STRENGTHS FROM HALF-SHORN SHEEP

Sheep No.	Staple Strength, lb/g			
	1954		1955	
	Pre-lamb	Post-lamb	Pre-lamb	Post-lamb
145	123	58*	160	78†
174	205	58*	188	71†
243	177	101	169	118
262	170	83†	188	139
Mean	169	75	176	102

* cotted and tender
† tender

OPENNESS OF FLEECES

A prime complaint of those who buy and handle pre-lamb-shorn wool is that the fleeces are more loose and open than those to which they are accustomed, so that they break up easily when handled. This makes them more difficult to sort to best advantage, although on the other hand some mills prefer pre-lamb-shorn fleeces because less physical effort is required in sorting the fleeces into matchings.

This aspect was watched during the classing of the fleeces after shearing, and it was observed that while the pre-lamb-shorn fleeces were in fact all relatively open, the post-lamb-shorn fleeces varied from some that were equally as open (mostly those graded as "good") to others that were hard cotts. It was apparent that the openness of the fleeces was closely related to their soundness, the good sound fleeces being open while faulty fleeces hung together more, the extreme being reached with cotts. Thus, conversely, the openness of a fleece is an indication of its degree of soundness.

DISCUSSION

The outstanding feature of the fleece results given above is the very large difference in the incidence of wool faults as between the pre-lamb- and post-lamb-shorn clips. In each year the pre-lamb-shorn

clips included only occasional fleeces showing slight tenderness or cotting and a few fleeces with mushy backs or coloured yolk. The post-lamb-shorn clips on the other hand included a large number of fleeces that were tender and/or cotted, about 80% of the fleeces in both flocks being so affected in 1956. Thus there was a very large difference between the two clips in each pair as regards soundness of staple, which is borne out by the actual strength tests given in Tables 4 and 5. As it happened, there was apparently a progressive deterioration in the soundness of the post-lamb-shorn wools through the four years at Invermay, though this may have been partly due to varying standards of grading. Apart from this, such differences are seasonal in character and depend mainly on the weather and availability of feed during the winter and early spring months. It is interesting to note that although the average fleece weights from both flocks were lower in 1956 than in the previous years, owing to poor autumn conditions, the pre-lamb-shorn clips were nevertheless sound while the post-lamb-shorn clips had the highest recorded proportions of tender fleeces.

It should be mentioned that a higher proportion of pre-lamb-shorn fleeces was down-graded for non-wool (acquired) faults, usually for dust or moit (vegetable matter — chaff, hay, or gorse), since these were almost the only faults: also, in 1955 both pre-lamb-shorn clips were rather muddy following a wet spell. Such faults, while detracting from the colour and style of the wool, do not affect its soundness, but may have some adverse effect on processing on account of the increased mechanical treatment that may be needed to remove the contamination

The fleece weights and lengths also show annual fluctuations, and there is no consistent difference from year to year between the clips shorn at the two different times. Thus there is no evidence to support the statement that pre-lamb shearing leads to shorter and lighter fleeces. In fact, the mean weight and length, taken over the 6 clips, was practically the same for the different shearing times (Table 1).

The pre-lamb-shorn Romney fleeces were more open and tended to break up more than the corresponding post-lamb-shorn fleeces. However it is considered that this is not a fault (except insofar as it makes the fleeces rather more difficult to handle and sort) but a result and indication of the soundness of the wool. A similar difference was not observed with the non-cotted Corriedale wools.

On the other hand the webbiness of post-lamb-shorn fleeces is likely to be an indication of weakness, since it probably represents the first stages of cotting. Thus to our way of thinking it is webbiness which is a fault, not openness.

This factor of openness may be further considered in relation to mill processing. If the wool is open, less mechanical action is needed to separate the fibres than if it is webby and holds together, and so less fibre breakage would be expected, leading to a better combing tear. This is apart from the superior strength of pre-lamb-shorn wool which should also lead to a better tear.

Conclusions

1. Pre-lamb-shorn wool has been found to be much superior to post-lamb-shorn wool in soundness and freedom from wool faults such as tenderness and cotting.

2. No evidence was found that pre-lamb-shorn fleeces of a full year's growth are consistently lighter or shorter than post-lamb-shorn, as has been frequently stated.

3. The openness of pre-lamb-shorn fleeces is an indication of the essential soundness of the wool.

Acknowledgements

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EFFECT OF SHEARING TIME ON WOOL

V. EFFECT ON PROCESSING

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Summary

Two further worsted processing trials have been made, using Romney (48/50s) and Corriedale (56/58s) wools. In each trial the manufacturing performances of pre-lamb-shorn and post-lamb-shorn wools from the same flock of sheep were compared. In both cases the pre-lamb-shorn wool gave a higher yield, less noil on Noble combing, a longer top, and a considerably stronger yarn, and thus had a superior performance in processing.

Introduction

The original object of the experiments was to see whether pre-lamb-shorn wool was inferior to post-lamb-shorn in its processing performance, as had been claimed by woolbuyers. The first processing trial on two lots of wool shorn at the two different times from sheep belonging to the same flock was made on Romney wool shorn at the Invermay Research Station, Mosgiel, in 1953, and was described in Part III of this series (Story 1955). In that trial little difference was found in noilage or length of top, but there was an appreciable difference in yarn strength in favour of the pre-lamb-shorn wool. However, it was known that there was an initial difference in staple length in favour of the post-lamb-shorn fleeces, which were slightly the longer, giving this lot some advantage and making it appear rather better than it otherwise would have been.

Similar processing trials have since been made on Romney wool shorn at Invermay in 1954, and on 56/58s Corriedale wool shorn at a farm near Waimate in 1955 (Story and Ross 1959). In the former, the two lots were of similar length, while in the latter the pre-lamb-shorn lot after sorting was still rather longer and of better style than the post-lamb-shorn, which would contribute to a superior performance.

Experimental

The processing procedure was similar to that used before, in each case the two lots of wool being processed one after the other on the same machinery, with observations and taking of samples for measurement at appropriate stages.

Selection of Wool for Processing

By examination of the fleeces immediately after shearing it had been found that those shorn before lambing were almost invariably sound, while those shorn after lambing were more or less tender,

depending on the season, 1955 and 1956 being worse than 1953 and 1954. From the point of view of comparing worsted processing performance it was first necessary to eliminate this gross difference between fleeces by selecting only fleeces or wool that appeared to be sound, and then seeing if a difference still arose during processing. Naturally, because of the very different incidence of wool faults in the two lots, the proportion of pre-lamb-shorn wool suitable for combing was considerably higher than that of post-lamb-shorn wool.

The procedure for selecting the 1954 Romney and the 1955 Corriedale wools was rather different, mainly because of the larger incidence of wool faults in the 1955 post-lamb shearing. At Invermay the Romney fleeces were classed according to quality and graded as "good" or "faulty", and the "good" 48/50s quality fleeces were used for the processing trial. The proportion of 48/50s quality wool was lower than it had been in 1953 because the fleeces were heavier and a little stronger in 1954. but the total 48/50s was about the same at the two 1954 shearings. The selected "good" fleeces represented 26.3% and 17.1% of the skirted weights of the pre-lamb- and post-lamb-shorn clips respectively. Actually a still higher percentage of the pre-lamb-shorn could have been used, as most of the "faulty" 48/50s shorn at that time were only affected by dust or moit, which would not have interfered with processing.

The position with the 1955 Corriedale wool was rather different in that a total of only 9% of the post-lamb-shorn fleeces was graded as free from faults, so that it was not possible to select a line of sound post-lamb-shorn fleeces for processing. Consequently it was necessary to take the whole of the two clips to the mill (with the exception of fleeces that were too fine or too strong), and to obtain lines for processing by sorting out the best parts of the available fleeces. The percentages of combing and carding wool obtained, calculated on the weights of the skirted fleeces, were as follows:

	Pre-lamb-shorn	Post-lamb-shorn
Combing	68.6	30.8
Carding, clear	12.2	63.9
Carding, seedy*	18.7	3.5
Oddments	0.5	1.8
	100.0	100.0

* A comment on this has been made in Part IV.

It will be seen that the percentage of wool suitable for combing obtained from the pre-lamb-shorn lot was more than double that from the other lot. In addition to the large difference in amount, it was observed that the pre-lamb-shorn combing lot had a rather better colour, appearance, and length than the other, the two lots being graded BB and B/BB respectively. This was unfortunate from the processing

point of view, as it gave the pre-lamb-shorn lot an advantage from the start, but it could not be avoided without rejecting more of the post-lamb-shorn wool and making a very small lot for processing. Looking at it another way however, it is significant that the post-lamb-shorn wool was vastly inferior to the pre-lamb-shorn, so that it was scarcely possible even by sorting to obtain matchings from the two lines.

In 1956 at Waimate, and also at Invermay, the proportions of fault-free post-lamb-shorn fleeces were again very small, and the same sorting procedure would have had to be adopted for processing trials, which however were not considered necessary in view of the results obtained with 1954 and 1955 wools.

Scouring Yield

This was determined as before by weighing the card sliver and waste and determining the mean regain of the sliver. From these data the weight of clean wool at 16% regain was calculated and expressed as a percentage yield on the initial weight of the greasy wool scoured. The yields obtained were

Wool	Pre-lamb-shorn	Post-lamb-shorn
Romney, 1954	75.0	72.3
Corriedale, 1955	68.2	66.5

Topmaking

In each case the two lots were processed one after the other on the same machines, except that the Romney wools were combed on two similar machines. The carding rates were 37 lb per hour for the Romney wools and 43 lb per hour for the Corriedale wools, and there was no difference in percentage of card waste between pre-lamb- and post-lamb-shorn lots. The Romney wools were backwashed after carding, but the Corriedale wools were gilled only; both wools were oiled with mineral-oil-based emulsions.

A careful check of the combing was made by stopping the Noble comb at intervals (timed by stop watch) to change the sliver can and remove the noil. The sliver and noil were weighed each time and samples taken for determination of the regain. Small adjustments were made as necessary to balance the production of the two small circles of the comb and keep the overall production fairly steady (the weight of wool available did not permit of starting with a full load on the comb). The Romney wools were combed on two identical machines running at the same speed except for a difference in speed of drawing-off leathers. To allow a correction to be made for this difference between the machines, portions of another lot of wool were subsequently combed on both machines under the same conditions and the percentages of noil calculated; these differed by only 0.3 in 8% due to the machine differences.

The Romney wools were combed once only, but the Corriedale wools were mothproofed after combing, backwashed, and re-combed, the same procedure as before being followed.

The tears and percentages of noil were calculated after making allowances for the differences in regain (and between combs), and the results are given in Table 1, from which it will be seen that the pre-lamb-shorn lots consistently gave the less noil and the better tear.

TABLE 1. COMBING TESTS

Wool	Combing	Production of sliver lb/hour	Tear		Noil, %	
			Lot A*	Lot B*	Lot A	Lot B
Romney	First	46	22.8	16.5	4.2	5.7
Corriedale	First	43	14.4	11.5	6.5	8.0
Corriedale	Second	85	64	49	1.5	2.0

* Lot A = pre-lamb-shorn; Lot B = post-lamb-shorn.

Spinning

The tops were drawn on Bradford open-drawing sets and in each case the rovings were spun simultaneously on the same machine. The tops and rovings were stored for a few weeks before drawing and spinning respectively.

Romney Wools. These were cap-spun to 1/24s worsted, using half of one side of a frame for each lot for ends down studies. At the first doff the pre-lamb-shorn lot had had only half the ends down of the other lot, but afterwards there was little difference between the lots.

Corriedale Wools. As the roving weights differed slightly, the two lots were spun on opposite sides of a frame with different drafts (slightly higher for pre-lamb-shorn). 1/26s worsted yarns were spun and no genuine ends down were observed as this count was well within the spinning limit of the wool quality. For a short time the frame was run at higher drafts and with rather soft twists to induce end breakages, and it was found that ends did then come down more frequently on the post-lamb-shorn side. For example, the finest yarn spun was approx 1/41s, the drafts being 12.4 and 12.0 for the pre- and post-lamb-shorn sides respectively; the ends down in 30 minutes, with 72 spindles per side, at a nominal twist of 12.7 t.p.i., were 18 and 40 for the pre- and post-lamb-shorn sides respectively. It is appreciated that the drafts are considerably higher than is customary and that it would have been better to have made a lighter roving, but on the other hand both lots were treated the same and at the same time so that it is considered that the results show a significant difference in favour of the pre-lamb-shorn wool.

Fibre Measurements

Measurements of fibre fineness (projection microscope) and fibre length (comb-sorter and WIRA fibre-length machine) were made on samples of scoured wool, gilled card-sliver ex punch-balls, tops, noil, and roving. The results are given in Table 2, from which it will be seen that there was little if any difference in fibre diameter between

TABLE 2. Fibre Measurements

	Romney		Corriedale	
	Lot A*	Lot B*	Lot A	Lot B
Fibre diameter, microns				
Card sliver	35.1	34.4	29.0	29.1
First top	37.0	36.0	29.1	28.8
First noil	27.4	29.2	23.7	23.5
Fibre length (comb-sorter), cm				
Card sliver			8.8	8.1
First top	13.9	12.8	9.7	9.1
Second top	—	—	10.5	9.8
Roving	14.0	12.9		
Fibre length (single fibres), cm				
Scoured wool	11.3	11.4	11.3	9.7
Card sliver	7.9	6.4	7.9	6.9
First top	11.2	9.9	9.0	8.1
First noil	1.8	1.8	2.1	2.3

* Lot A = pre-lamb-shorn; Lot B = post-lamb-shorn.

the two lots from the same source, but there were considerable differences in fibre length in samples of card sliver and top, the pre-lamb-shorn being longer than the post-lamb-shorn. The low figures recorded for the fibre length of the Romney scoured wools and card slivers as compared with the Corriedales were due to the large number of very short pieces of fibre encountered in measuring the Romney samples. Thus an average of 22% of fibres less than 3 cm long was recorded for the Romney samples, compared with only 9% for the Corriedales.

Yarn Tests

Single-thread strength tests (10 tests per bobbin on 10 bobbins per lot per doff of Romney wools and 6 bobbins per lot per doff of

TABLE 3. Yarn Tests

	Romney		Corriedale	
	Lot A*	Lot B*	Lot A	Lot B
Single-thread tests:				
Strength, oz	6.6	5.6	5.1	4.2
Extension, %	12.5	10.2	10.0	7.8
Regularity, C.V. %	26.4	27.2	18.4	19.6
Hank tests:				
Strength, lb	43.9	35.5	41.2	33.2
Count, worsted	23.4	23.6	25.3	25.3

* Lot A = pre-lamb-shorn; Lot B = post-lamb-shorn.

Corriedale wools) and hank strength and count measurements (one 120-yd hank per bobbin) were made as before in the conditioning room at 68°F and 65% r.h. Regularity tests were also made using an Uster testing apparatus. The mean results of the tests are given in Table 3, and show a large difference in strength in favour of the pre-lamb-shorn yarns, which were also the more regular.

DISCUSSION

There are two principle aspects of the worsted processing of the wools that are vitally related to the different shearing times of the two lots of wool in each of the trials. These are firstly the proportion of the total clip that was suitable for worsted processing, and secondly the differences found during the processing.

Suitability for Combing

On account of the much lower incidence of serious wool faults it has been found that the proportion of the clip suitable for combing is considerably higher for the pre-lamb-shorn wool; in fact, discounting the presence of dust and a little moit (which are extraneous to the wool though they affect its appearance and sale value), the bulk of the pre-lamb-shorn clip is suitable for combing. On the other hand much of the post-lamb-shorn clip has been found to be tender and so unsuitable for combing. The proportion of unsound wool varied considerably from year to year owing to differences in weather conditions, etc.

In the actual mill trials, the two lots for processing were selected as being the best of the wool of the required quality (fineness). Nevertheless there were still some differences between them. In the first trial with Romney (Invermay) wool (Story 1955, Part III) the pre-lamb-shorn wool was a little shorter than the other but otherwise there was no noticeable difference after sorting, while in the second trial with Romney wool the two lots were to all appearances identical. In the case of the Corriedale wools the post-lamb-shorn clip was so inferior that it was not even possible to sort from it a lot that had as good an appearance as the corresponding pre-lamb-shorn lot, consequently in this case the superiority of the pre-lamb-shorn combing lot was fairly evident even before the commencement of processing. These differences are reflected in the processing results.

Processing

In each manufacturing trial the pre-lamb-shorn lot has given the better performance. Thus there has been a higher scouring yield, a better tear, and less noil in combing it (in the first trial with Romney wool (Part III) there was a negligible difference in tear but the two lots started with the post-lamb-shorn being the longer which should have led to a better tear, other things being equal), a longer fibre length in the top, some evidence of better spinning performance, and a large difference in yarn strength.

It is evident that although at the commencement of processing only the best of the wool was selected and there was then little or no apparent difference between the selected pre-lamb-shorn and post-lamb-

shorn lots, yet there must still have been a considerable intrinsic difference between the two lots. This led to a smaller fibre breakage in the pre-lamb-shorn lot, resulting in less noil to be extracted, a longer top, and a considerably stronger yarn.

These trials were carried out to see if there was any basis for claims that pre-lamb-shorn wool gave an inferior worsted combing performance. They have been made with wools of 48/50s and 56/58s qualities, and in each case the results have completely disproved such claims, and have in fact shown that the pre-lamb-shorn wools of 12 months' growth have a considerably better processing performance, besides comprising a higher proportion of the original clip.

Conclusions

When two lots of apparently similar combing wool, one shorn before lambing and the other from another part of the same flock after lambing, are manufactured into yarn, the pre-lamb-shorn lot has a considerably superior processing performance. The results of the trials that have been made completely disprove claims that the pre-lamb-shorn wool is inferior in performance.

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THE EFFECTS OF FEEDING WHEYS OF DIFFERENT AGES TO FATTENING PIGS

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Summary

Pigs were fed a fixed daily allowance of ½ lb barley meal and ½ lb meat meal. Whey was rationed to individual animals to give a total daily dry matter (d.m.) intake equal to 3.5% of body weight.

Three different wheys were tested:

(a) Whey obtained daily from a commercial cheese factory.

(b) Whey from the same source kept to simulate a supply received on four days a week.

(c) Whey from the College cheese factory received on four days a week.

Additional treatments were included in which the wheys were treated with bleaching powder in order to reduce acidity development.

In the spring trial (50–120 lb live weight) growth rates and food conversion efficiencies were very similar for all groups. The only significant difference was a greater prevalence of scouring from about 80 lb l.w. on the College whey.

In the summer trial (50–110 lb l.w.), compared with the whey received daily, whey obtained on four days a week significantly increased fattening time by 5–6 days, and total food consumption by 15–17 lb d.m., approximately 9%.

Treatment of the wheys with bleaching powder reduced their mean acidity level but had no effect on the growth rate or food economy of the pigs.

Introduction

Over the years, supplies of milk for cheese manufacture in New Zealand have been organised on the basis of the daily delivery of milk to dairy factories by individual farmers. In the case of suppliers making use of the whey for pig production, the normal procedure is to return from the factory with whey derived from the previous day's cheese making. Partly as a matter of convenience, this has led to the practice of not feeding whey until it is about 24-30 hours old; and, normally, the supply is used up over the subsequent 24 hours. The view is widely held that to depart from this routine and to feed whey of variable age results in scouring and reduced growth rate, especially in the case of pigs of less than porker weight.

The College pig unit draws its whey supplies from the College dairy factory at which cheese is usually made on only four days each week. The question of the extent to which changes in the whey caused by this non-daily supply might affect the performance of fattening pigs was considered to be of importance in relation to our whey feeding investigations.

The reasons for the alleged adverse effects of feeding whey of variable age are not well understood but variability in the acidity of the

whey is a possible contributory factor. In planning our trials the opportunity was therefore taken to obtain some information on the effect of reducing natural acidity development by the addition of a hypochlorite.

During the spring and summer of 1957/8, two trials were carried out in which the effect of feeding from a regular daily supply of whey was compared with feeding from supplies available on only four days each week, both with and without the addition of bleaching powder to minimise acidity development.

Method

Trial I was carried out from the end of August to early December 1957. Trial II commenced in early December and continued to March 1958.

Experimental Design and Animals Used

In Trial I a randomised block layout was adopted, consisting of four treatments and seven replications. In Trial II a 3 × 2 factorial design was used with five pigs on each of four treatments and with four pigs on each of the remaining two treatments.

With the exception of one block of purebred Large White pigs in each trial, the pigs used were Large White × Berkshires. Blocks consisted of litter mates selected at 40–45 lb live weight for similarity in weight and previous growth rate. Allocation to treatments within blocks was at random with the restriction that the treatment groups were balanced for sex as far as possible.

Supplies of Whey

For the purpose of these trials, whey was obtained from two sources, viz, from the College dairy factory and from the Kairanga Dairy Co. cheese factory at Aokautere, about 4 miles away.

Supplies from the former were pumped to the piggery within 3 hours of being "run" from the vats and were stored in a feed room in steel tanks and drums. Whey supplies from the Aokautere factory, derived from the previous day's cheese making, were delivered each morning in 20-gallon milk cans which on arrival were placed in the same room as the whey from the College factory.

Experimental Treatments

In Trial I the four treatments were:

I—Control. Whey from the Aokautere factory, first fed when approximately 24 hours old and with supplies renewed daily (designated AF whey).

II—Whey from the same source as I but held over for feeding as necessary to stimulate the four-day per week supply as available on Treatment III (designated AO whey).

III—Whey from the College factory supplied on four days weekly, i.e. on Mondays, Tuesdays, Thursdays and Fridays (designated C whey).

IV—As for III but with the addition of bleaching powder (designated C + Bl whey).

In Trial II the above treatments were repeated with the addition of two further treatments consisting of AF and AO wheys to which bleaching powder had been added: that is, a comparison was made between the three main treatments with and without the addition of bleaching powder in all cases. Because accommodation limited the number of pigs that could be used to 28, in this trial the two treatment groups receiving whey from the College factory each contained only four pigs.

Treatment of Wheys

In view of day-to-day variation in the dry matter (d.m.) content of the wheys, both between and within the two sources of supply, it was necessary to equalise d.m. contents by dilution of the more concentrated whey with water. Since some of each day's supply of whey from the outside factory was needed for feeding within three hours of its delivery to the piggery, the normal oven-drying method for estimating d.m. content could not be used. Accordingly, a density method (using approximately two litres of whey) was developed for the purpose (McDowall and McDowell pers. comm.). The results obtained by this method were checked against those obtained by the normal method on several occasions during the trial. In most cases differences were within the range 0.05–0.15% d.m., the greatest individual difference recorded being 0.25% d.m.

Records from the first trial showed that although daily variation in d.m. could be substantial, over a period the mean within-factory figures were fairly uniform, with the College whey having a consistently higher d.m. content than the Aokautere whey. Accordingly, for the second trial the College whey was routinely diluted with sufficient water to reduce the d.m. content from an assumed level of 6.45% to one of 6.15%. Daily d.m. estimations by the density method were replaced by weekly estimations, by conventional methods, of composite samples consisting of bulked 20 ml samples taken daily from each of the three untreated wheys.

Bleaching powder was added in the appropriate treatments within 3–4 hours of the whey being "run" from the cheese vats. In Trial I, 5 oz of a commercial hypochlorite (containing approximately 30% available chlorine) was added per 100 gal whey. In Trial II, when hotter weather was experienced, this rate was doubled.

The acidities of the wheys were estimated by titration against 0.1 N sodium hydroxide. Titrations were carried out each morning, both on the wheys used in the previous 24 hours and on those to be used in the succeeding 24-hour period.

Details of Feeding

All pigs received a fixed daily meal allowance consisting of $\frac{1}{2}$ lb barley meal and $\frac{1}{2}$ lb meat meal (minimum 57% crude protein). Whey was fed three times daily at 6.30 a.m., 11.30 a.m., and 4 p.m. Fresh supplies of whey were used for the first time at the 11.30 a.m. feed.

In the case of the treatment in which regular daily supplies of whey were used, a new supply was started at each 11.30 a.m. feed. The remaining treatments differed to the extent that whey produced on Tuesday was fed from 11.30 a.m. on Wednesdays to 6.30 a.m. on Fridays, while that produced on Fridays was fed from 11.30 a.m. on Saturdays up to and including the 6.30 a.m. feed on the following Tuesday.

The daily allowance of whey for each pig was rationed according to a scale designed to give a total daily d.m. intake equivalent in weight to 3.5% body weight, assuming d.m. contents of 87% for barley meal, 91% for meat meal, and 6% for whey. Any whey refusals were weighed back prior to the morning feed.

Once weekly all pigs received 10 g of a Vitamin A and D_3 emulsion containing 5000 i.u. of Vitamin A and 500 i.u. of Vitamin D_3 per gramme.

General Management

In both trials the pigs were kept in individual pens in a new, well-insulated, totally enclosed piggery of the Danish type equipped with an air-conditioning unit that maintained the temperature within fairly narrow limits, by heating or cooling the incoming air. The mean air temperature was 68°F. Although some initial troubles were experienced with the air-conditioning equipment, the temperature rarely exceeded the range 60–70°F. In Trial I the temperature fell to between 50° and 60°F for about 6% of the time and in Trial II for 3% of the time. No bedding or drinking water was provided.

The pigs were placed individually on their experimental rations as they reached a live weight nearest to 50 lb. Throughout the trial they were weighed twice weekly, before the morning feed, and the necessary adjustments were made to their daily allowances of whey. Treatments were continued until all pigs weighed at least 120 lb live weight in Trial I and at least 110 lb in Trial II.

Observations on the consistency of the dung of each pig were made once daily, a subjective classification comprising three grades—"normal", "soft", and "scour"—being used.

Results

D.M. Content of Wheys

Data relating to the d.m. content of the wheys used are presented in Table 1.

From the results for Trial I it is seen that the Aokautere whey was lower and more variable in d.m. content than the College whey. In Trial II, after the College whey had been diluted daily with a fixed proportion of water, the mean d.m. content was very similar to that of the Aokautere whey, but, again, it was less variable.

Titratable Acidity of Wheys

The results given in Table 2 cover the period from the time half the pigs had reached 50 lb live weight until the time half had reached

TABLE 1. D.M. CONTENTS OF WHEYS

	Trial I* Wheys as received		Trial II** Wheys as fed		
	C	AF	C	AF	AO
Mean d.m.	6.54	6.06	6.19	6.22	6.25
Standard deviation	.09	.19	.08	.15	.12
Range	6.29–6.69	5.44–6.34	6.07–6.30	5.98–6.40	6.09–6.46

* daily estimations by density method.
** weekly composite samples estimated by normal method.

TABLE 2. TITRATABLE ACIDITIES OF WHEYS
(expressed as percentage lactic acid)

Whey	Trial I		Trial II	
	Mean Acidity %	Standard Deviation	Mean Acidity %	Standard Deviation
AF	.40	.06	.51	.08
AF + B1	—	—	.24	.06
AO	.42	.09	.58	.12
AO + B1	—	—	.33	.11
C	.27	.06	.43	.07
C + B1	.20	.05	.29	.10

120 lb. In calculating these results the means of the titrations taken at the beginning and the end of each 24-hour feeding period were used.

The general level of acidity was higher in the second trial than in the first. In both trials there was little difference between the mean acidities of the untreated Aokautere wheys (AF and AO), though as would be expected, the AO whey showed greater day-to-day variation. The non-daily supply of whey from the College factory (C) had a lower mean acidity, and was not subject to any greater variation than the whey supplied daily from Aokautere (AF). In both trials, the addition of bleaching powder reduced the mean acidity levels but had little effect on variability except in the case of the College whey in Trial II, when variation was somewhat increased.

Performance of Experimental Animals

Trial I. One pig on Treatment II (AO whey) died from strangulation of the gut shortly after the trial started. A second pig, on Treatment I (AF whey), developed prolapse of the rectum at a later stage and had to be removed. All other pigs remained in good health.

Analyses of variance were carried out on the number of days required to reach 120 lb live weight, on the amount of whey consumed during the period and on the total d.m. intake. (Since 1 lb of meal was fed daily, the meal-consumption figures were identical with the fattening time expressed in days.) Values calculated by missing plot technique were used in the case of the two casualty pigs.

A summary of the results is presented in Table 3.

TABLE 3. Trial I—Summary of Growth, Food Consumption, and Food Economy

	Whey Treatments				Signif. of Diff.	Error Coeff. of Variation
	I* AF	II* AO	III C	IV C + Bl		
Initial live wt	50.0	50.0	50.1	50.0		
Days to 120 lb	71.1	71.0	74.1	72.7	N.S. (F=1.04)	5.3%
Weekly gain (lb)	6.9	6.9	6.6	6.7		
Meal (lb)	71	71	74	73	N.S. (F=1.04)	5.3%
Whey (lb)	2304	2305	2410	2327	N.S. (F=1.14)	5.3%
Total d.m. (lb)	203.0	203.1	212.1	205.9	N.S. (F=0.88)	5.5%
Meal/1 lb gain (lb)	1.01	1.01	1.06	1.04		
Whey/1 lb gain (lb)	32.9	32.9	34.5	33.2		

* Missing plot value calculated for 1 pig.

The two types of whey supply from the Aokautere factory (AF and AO whey treatments) gave identical results for length of fattening time, and for meal, whey, and total d.m. consumption. The C and C + Bl whey treatments were slightly but not significantly inferior. The error coefficient of variation was uniformly small in all variance analyses.

The incidence of scouring appeared to vary between treatments and at different stages of growth. Table 4 shows the average percentage of occasions on which scouring was recorded for the various experimental treatments during four successive stages of growth.

Up to 65 lb live weight there was a moderate amount of scouring on all treatments. Subsequently, the AF and AO whey treatments showed little further increase, but there was progressively more scouring on the C whey treatment and an even higher incidence on the C + Bl treatment at heavier weights. Except for the period up to 65 lb, chi-square values calculated for individual periods and for the overall period were all in excess of a probability of $P = 0.01$, while a significant interaction between periods (excluding period 50–65 lb) and treatments was found, indicating that scouring was not randomly distributed

TABLE 4. TRIAL I—INCIDENCE OF SCOURING
(expressed as percentage of total observations)

Treatment	AF	AO	C	C + Bl	Mean
50– 65 lb l. wt.	17.0	14.7	23.2	24.2	20.1
66– 80 lb l. wt.	17.5	4.5	13.7	36.9	18.6
81– 95 lb l. wt	18.4	7.1	38.7	42.5	27.2
96–120 lb l. wt.	25.3	13.4	52.5	61.3	39.5
Mean	19.4	10.3	32.3	40.6	26.3

either between treatments within growth periods or between periods. On the other hand, there was considerable individual variation within treatments. Thus, the incidence of scouring in individual pigs ranged from 5–33% on AF whey, 0–14% on AO whey, 0–48% on C whey, and 3–72% on C + Bl whey.

Trial II. As in Trial I there were two casualties. One pig on the AO + Bl treatment developed a prolapsed rectum at 65 lb and was removed. One pig on the AF treatment became unthrifty and grew so slowly and erratically from the fifth week of the trial that its results were discarded. Apart from one other pig, on the AO treatment, which was off food for two days towards the end of the trial, there were no other ailments and no significant food refusals.

Because of the unequal number of pigs on the various treatments, analyses of the growth and food consumption data were undertaken by the method for disproportionate sub-class numbers outlined by Snedecor (1946). In the case of all four criteria—number of days required to reach 110 lb live weight, meal, whey, and total d.m. consumption—preliminary analyses showed that there was negligible interaction. Differences among the treatment means were tested for significance by the method of fitting constants. In all analyses, the F test indicated that significant differences existed among the means for whey treatments. Differences between individual whey treatments were tested for significance by the multiple range test of Duncan (1955). The F test showed that the means for the bleaching powder treatments did not differ significantly.

Results are summarised in Table 5.

Considering the three main whey treatments, by comparison with the daily supply (AF), the non-daily supplies (AO and C) in both cases resulted in an extension of the fattening time by 5–6 days (8.8%), the differences being highly significant. Meal, whey, and total dry-matter consumption all showed a similar trend, whey consumption being 16–20 gal greater and total d.m. intake 15–17 lb greater in the case of the groups receiving the non-daily whey supplies.

The results for pigs receiving wheys treated with bleaching powder were virtually identical with those of pigs fed the untreated wheys.

TABLE 5. Trial II—Summary of Growth, Food Consumption, and Food Economy

Comparison between Wheys

	Whey Treatment			Signif. of Differences	Error Coeff. of Variation
	I AF	II AO	III C		
No. of pigs	9	9	8		
Initial live weight	50.3	50.1	50.1		
Days to 110 lb	62.7	68.2	68.9	AF v. AO, C-P <0.1	5.8%
Weekly gain (lb)	6.66	6.15	6.09		
Meal (lb)	62.7	68.2	68.9	AF v. AO, C-P <.01	5.8%
Whey (lb)	1906	2063	2106	AF v. C, -P <.01 AF v. AO, -P <0.5	8.6%
Total d.m. (lb)	174.7	189.7	191.5	AF v. AO, C-P <.01	5.7%
Meal/1 lb gain (lb)	1.05	1.14	1.15		
Whey/1 lb gain (lb)	31.9	34.4	35.2		

No Bleaching Powder v. Bleaching Powder

	No. Bl. Powder	Bleach. Powder	Signif. of Differences
No. of pigs	13	13	
Initial live weight	50.0	50.3	
Days to 110 lb	66.7	66.3	N.S.
Meal (lb)	66.7	66.3	N.S.
Whey (lb)	2017	2027	N.S.
Total d.m. (lb)	184.9	185.2	N.S.

A study of the observations on the consistency of the dung disclosed extremely wide differences between individual pigs on all treatments. Rather marked differences also occurred between blocks, indicating that some litters were more prone to scouring than others. However, because one or two animals contributed the majority of the "scour" observations on some treatments, there appeared to be little point in analysing the data statistically.

Discussion

The reason for the consistently higher d.m. content of the whey from the College dairy factory may be found partly in the fact that at the College factory the washing water was not pumped to the whey

holding tank as is customary at commercial cheese factories. Some additional dilution of the Aokautere whey occurred from rain falling in the uncovered factory whey tank. Invariably after heavy rain the d.m. content of the latter whey was below the mean value, occasionally falling as low as 5.4–5.8%.

The lower acidity of the College whey was probably due primarily to the cooling that occurred as a result of its passage through the half-mile length of underground supply pipe running from the factory to the piggery.

In general, the performance of pigs in both trials was satisfactory, bearing in mind the stage of growth covered and the level of meal supplementation.

In the first trial the greater prevalence of scouring on the C and C + Bl whey treatments may have been a factor contributing to the slightly slower growth of these groups.

The superiority of the daily supply of whey (AF) in Trial II was in contrast to the identical results for daily and non-daily Aokautere wheys obtained in Trial I. No obvious reason for this difference is apparent but since the latter trial was carried out during the summer, some factor associated with the hotter weather is suggested. It seems unlikely that the level and/or variability of the titratable acidity of the wheys *per se* were responsible since these characteristics in the AF whey, which gave the best results, were intermediate between those for the AO and C wheys.

Treatment with bleaching powder was effective in reducing the mean acidity levels of the wheys but the quantities used were insufficient to reduce day-to-day variation in the non-daily supplies. Possibly greater quantities of hypochlorite could have been used without affecting the pigs adversely. Whitehead and Hunter (1947) reported that pigs fed whey containing up to three times the level of bleaching powder used in Trial II showed no ill effects but this high rate was used for only a very short period.

It is open to question whether the differences in mean acidity and variability between the different wheys were sufficiently great in our trials to be of any critical importance. Within the comparatively narrow range of acidity occurring in these trials there was no evidence that a low acidity was of any advantage. On the contrary, for Trial I the relative incidence of scouring on the four treatments was in inverse relationship to the mean acidity levels of the wheys. However, undue emphasis should not be given to this point in view of the indefinite nature of the results obtained in Trial II on the incidence of scouring. Because of the wide differences existing between individual pigs, and to a lesser extent between litters, in respect of proneness to scour, it is clear that in order to make a satisfactory study of the possible relationship between scouring and various dietary factors trials involving large numbers of pigs are required.

Taken together, the results of the two trials indicate that, by comparison with a daily supply of whey, a four-day-per-week supply can

have an adverse effect on growth rate and food economy, although differences may be negligible during the cooler months. However, the differences obtained were not sufficiently marked to suggest that investigation of most whey-feeding problems would be seriously compromised by the use of whey supplied on only four days each week.

The addition of bleaching powder, at the rates used, had no beneficial effect on growth rate or on the incidence of scouring.

The wider implication of the results is that, while the customary practice of feeding from supplies of whey that are renewed daily is to be preferred to a four-day per week supply, the advantage may not be great. It appears that some flexibility in the delivery of whey could be justifiable if, for other reasons, this was expedient. For example, the introduction of the bulk collection of milk from farms could stimulate interest in non-daily delivery of whey. Frequency of delivery has an important bearing on cartage costs which in turn vitally affect the profitability of feeding whey to pigs once individual farmers no longer deliver their own milk to the factory daily.

Non-daily tanker delivery of whey might be one method of helping to solve the growing problem of disposing of the large quantities of unwanted whey that have been associated particularly with casein production in recent years. It is possible that, owing to the higher temperature to which it is subjected and its higher initial acidity, casein whey would undergo less rapid subsequent change in composition and bacterial flora than cheese whey, and in consequence be more suitable than the latter for feeding at variable age. Information is needed on the effect of longer intervals between delivery of supplies than those adopted in the present trials on the feeding value of both cheese and casein wheys, and on the possible need for added preservatives where supplies are delivered less frequently.

Acknowledgements

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TREATMENT OF NEW-BORN PIGS WITH IRON DEXTRAN

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Summary

An experiment has been described in which weekly recordings were made of the haemoglobin levels and weight gains of piglets from birth to weaning at eight weeks.

Half the group received two ml iron-dextran equivalent to 100 mg of iron on the day of birth and again at ten days of age. The remainder served as controls.

The administration of this compound prevented the marked fall in haemoglobin suffered by the control piglets but in no way affected the weight gains.

Under conditions in which piglets have access to pasture and soil, the use of iron dextran to overcome the lag in weight gain shown by many piglets in their second and third week is not recommended.

Introduction

The majority of suckling litters in New Zealand have access to pasture and soil throughout the period from birth to weaning. At this Station all litters are put out to pasture at two days of age, and under these conditions it would appear unlikely that farm- or Station-reared litters would normally suffer from nutritional anaemia. Examination of weekly weight gains, however, has shown that many litters fail to grow as rapidly in the second and third week of life as in the first and fourth week. At the same time, preliminary examinations indicated a fall in haemoglobin levels over the critical period.

Whilst other possible causes of this reduction in growth rate are currently being investigated, it was considered advisable to explore the possibility that sub-clinical anaemia was the main factor involved, and at the same time to establish whether or not any basic correlation existed between haemoglobin levels and growth rates.

Materials and Methods

Six litters of pigs comprising a total of 48 piglets were divided at the birth of each litter into two groups. One group was treated with iron dextran and the other group left as untreated controls.

Each piglet was bled from the ear at birth and thereafter at weekly intervals until weaning at eight weeks. Haemoglobin estimations were carried out on each blood sample by an alkaline haematin method.

The treated groups were injected intramuscularly immediately after the first bleeding with two ml of iron dextran equivalent to 100 mg iron, and the dose repeated after ten days.

All piglets were weighed weekly at the time of bleeding.

Results

There was a significant difference in haemoglobin levels between the treated and untreated groups at the two to three week period without any accompanying difference in live-weight gains. The average weekly haemoglobin levels and live-weight gains of both groups are shown in Figs. 1 and 2.

While the mean weekly weight gains for all litters shown in Fig. 2 does not indicate any reduction in growth rate during the second or third weeks of lactation, two of the six litters did, in fact, show a check in growth over this period. Both the treated and untreated animals exhibited the same reduction in rate of gain.

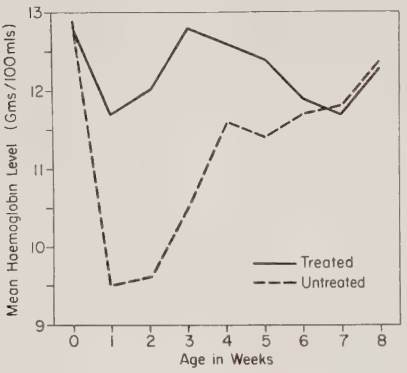

Fig. 1

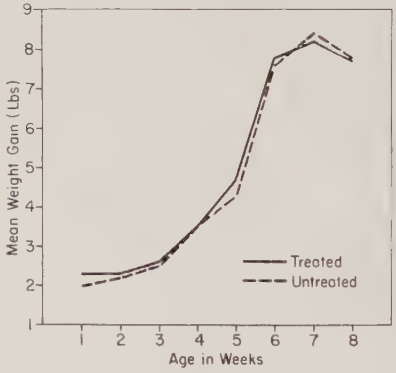

Fig. 2

Fig. 1.—Mean haemoglobin levels.

Fig. 2.—Mean weight gains.

Discussion

Whilst the experiment was primarily designed to examine a situation existing on this Station, it is known that many commercial pig breeders are accustomed to feeding or dosing piglets with some form of iron during their suckling period as a precaution against anaemia. The recent availability of easily assimilated iron dextran compounds has stimulated interest in such precautionary measures. These compounds are, however, relatively expensive, and under the conditions of this experiment would appear to serve no useful purpose for routine treatment of new-born piglets.

Acknowledgements

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THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

VII. THE THYSANOPTERA AS REVEALED BY SUMMER SWEEP-SAMPLING

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(*Received for publication, 7 August 1959*)

Summary

A sweep-sampling survey during the summer of 1957 revealed 12 species of Thysanoptera in the main pasture types occurring in the North Island. The identity, distribution, and abundance of these species are indicated, and their possible economic importance is discussed.

Introduction

During the summer of 1957, a survey of insects associated with pastures in the North Island was carried out. In the first paper of the series (Cumber 1958) techniques, coverage, and relevant sampling station details are given, and the insect complex generally as shown by sweep-sampling is analysed and discussed. Subsequent papers have provided information in respect of the Hemiptera, Coleoptera, Hymenoptera, Psocoptera and Neuroptera (Cumber 1959a, 1959b, 1959c, 1959d) and Diptera (Cumber and Harrison 1959).

The present account discusses the Thysanoptera with regard to the identity of the species, their distribution, numbers encountered, and possible economic importance.

The survey involves 113 paddock and 108 adjacent roadside samples each comprising a single sweep from an area of approximately 8 square yards.

Distribution maps for 7 species are given, a black dot indicating the presence of the species in contrast to a ring where sampling failed to reveal it.

Results

Sub-order **Terebrantia**

Fam. Aeolothripidae

T. 4 *Aeolothrips fasciatus* (L.)

Distribution

This cosmopolitan species was first recorded in New Zealand by Cottier (1931) who recorded its presence on potato from widespread localities in both Islands. Doull (1956a) records it from cocksfoot. In the present survey it was taken in small numbers in paddock samples from Tolaga Bay 12.2.57, Wairoa 12.2.57, and Waikohu 13.2.57, and in roadside samples from Puketona 18.1.57, Piopio 24.1.57, Tolaga Bay 12.2.57, Waikohu 13.2.57, and Okere 4.3.57.

Habits and Economic Significance

This species is predaceous on soft-bodied insects belonging to a number of orders, so probably plays a minor beneficial role.

Fam. Thripidae

T. 9 ? *Anaphothrips obscurus* Muller

Distribution

This appears to be the species first recorded from New Zealand by Doull (1956a), but the identity is by no means certain. It was taken in 92 paddock and 71 roadside samples and is seen to be present throughout the North Island (Fig. 1).

Habits and Economic Significance

Doull (1956a) records this species from cocksfoot at Lincoln. In the present survey it was taken in January (58 samples), February (42), March (60), and early April (3). In some samples numbers in excess of 200 were taken, and until its habits are better known it must be viewed with some suspicion.

T. 8 *Anaphothrips secticornis* Tyrbom

Distribution

This species was first recorded from New Zealand by Spiller (1951). In the present survey it was taken in 62 paddock and 47

Fig. 1

Fig. 2

Fig. 1.—Distribution map for the thripid ? *Anaphothrips obscurus* T 9.

Fig. 2.—Distribution map for the thripid *Anaphothrips secticornis* T 8.

roadside samples, sometimes occurring in considerable numbers. It is of widespread occurrence in the North Island (Fig. 2).

Habits and Economic Significance

Spiller's records (1951, 1956) indicate that citrus, dahlia, and pumpkin are hosts. In the present survey it was taken in January (34 samples), February (30), March (35), and early April (5). Until its habits are better known its importance in pastures cannot be gauged.

T. 10 ? *Aptinothrips rufus* Gmelin

Distribution

This cosmopolitan species was first recorded from New Zealand by Doull (1956a) when studying the thrips infesting cocksfoot at Lincoln. In the present survey it was taken in 76 paddock and 64 roadside samples from localities throughout the North Island (Fig. 3).

Habits and Economic Significance

Doull (1956a) indicates that this species "overwinters in the larval and adult stages on grass roots or at the base of the plant. Pupae are found on the foliage from October to May. Eggs are laid from September onwards. Sharga (1933) states that the whole life cycle occupies about 40 days and that the species is multivoltine." Doull and Sharga consider that this species may well be responsible for damage to grass seed crops. In the present survey it was taken in January (48 samples), February (32), March (56), and early April (4).

Fig. 3.—Distribution map for the thripid ? *Aptinothrips rufus* T 10.

Fig. 4.—Distribution map for the thripid *Chirothrips manicatus* T 6.

T. 6 *Chirothrips manicatus* Haliday

Distribution

This cosmopolitan species was first recorded from New Zealand (Auckland) by Spiller (1951). In the present survey it was taken in 44 paddock and 60 roadside samples often occurring in moderate numbers. It is of widespread occurrence in the North Island in areas south of Auckland (Fig. 4), and is probably widely distributed in the South Island.

Habits and Economic Significance

Doull (1956c) indicates that this species is common on meadow foxtail at Lincoln. Its widespread occurrence in North Island pastures indicates that it is in all probability associated with additional grasses. In the present survey it was taken in January (20 samples), February (27), March (54), and early April (3).

T. 12 *Chirothrips pallidicornis* Priesner

Distribution

This cosmopolitan species has been studied by Doull (1956b) under the name *C. manicatus,* this being corrected in a later paper (Doull 1956c). He records it from Canterbury. In the present survey it was taken only at Ohakune on 4.3.57.

Habits and Economic Significance

Detailed studies on the "cocksfoot thrips" by Doull (1956b) provide considerable information on the biology and life history of this species. He leaves little doubt as to its importance in South Island cocksfoot seed crops, yields in some instances being reduced by as much as 30%.

T. 5 *Heliothrips haemorrhoidalis* Bouché (Greenhouse Thrips)

Distribution

This cosmopolitan species appears to have been first recorded from New Zealand by Muggeridge (1935). Spiller (1951, 1956) records it from Kerikeri, Auckland, Little Barrier Island, Tauranga, and Palmerston North. In the present survey small numbers were taken in samples from Puketona 18.1.57 (roadside), Hikateruru 6.3.57 (paddock), and Tarata 2.4.57 (roadside). It would thus appear to be widely distributed in the North Island.

Habits and Economic Significance

Although this species is not of importance in pastures it is a pest of horticultural crops. Spiller records apple, citrus, cyclamen, *Fuchsia, Luculia, Mandevilla,* passion fruit, pumpkin, plum, viburnum, and walnut as host plants.

T. 11 ? *Isoneurothrips obscuratus* Crawford

Distribution

I. obscuratus was described by Crawford (1941). In the present survey, specimens of what is apparently this species were taken in a paddock sample from Whangaroa on 17.1.57. Spiller (1951, 1956) records it from widespread localities in the North and South Islands.

Habits and Economic Significance

Spiller records the following host plants—apple, boysenberry, *Buddleia,* canna lily, Chinese gooseberry, citrus, cyclamen, *Doryanthes, Fabia,* feijoa, *Freycinetia,* grape, hemlock, marigold, manuka, nikau, peach, *Phormium,* plum, *Pomaderris,* potato, *Protea,* puriri, rhododendron, tobacco, and wattle. It is not of importance in pastures.

T. 7 *Thrips tabaci* Lindeman (Onion Thrips)

Distribution

Cottier (1931) refers to the widespread occurrence of this cosmopolitan species in New Zealand. Spiller (1951) and Doull (1956a) record it from Auckland, Pukekohe, Palmerston North, Gisborne, Motueka, and Christchurch. In the present survey it was taken in 40 paddock and 41 roadside samples and is shown to be widely distributed in the North Island (Fig. 5). Occasionally it occurred in moderate numbers.

Fig. 5.—Distribution map for the thripid *Thrips tabaci* T 7.

Habits and Economic Significance

Cottier records this species as a pest of potato, swede, parsnips, peas, lucerne, French beans, and onions. It is recorded by Spiller and Doull as occurring on cocksfoot, chrysanthemum, marigold, onion, potato, sowthistle, strawberry, and tobacco. In the present survey it was taken in January (36 samples), February (14), March (30), and early April (1). Its importance in pastures cannot be gauged without further studies.

Sub-order **TUBULIFERA**

Fam. PHLOEOTHRIPIDAE

T. 1 *Haplothrips niger* Osborne

Distribution

This cosmopolitan species was first recorded from New Zealand by Muggeridge (1933). It is known to be widely distributed throughout both Islands. In the present survey it was taken in 31 paddock and 30 roadside samples (Fig. 6).

Habits and Economic Significance

Yates (1952) and Doull (1949) give detailed information on the biology and life history of this species in studies on its association with red clover in Nelson, Marlborough, and Canterbury. Yates estimates that it may be responsible for reductions of up to 20% in red-clover seed yields. Doull (1956a) also records white clover and *Plantago* spp. as hosts. In the present survey it was taken, occasionally in moderate numbers, in January (18 samples), February (23), March (18), and early April (2).

T. 2 ? *Haplothrips* sp.

This species was taken in 27 paddock and 17 roadside samples, from widely distributed parts of the North Island (Fig. 7). It is apparently an unrecorded species. It occurred, occasionally in moderate

Fig. 6

Fig. 7

Fig. 6.—Distribution map for the phloeothripid *Haplothrips niger* T. 1.

Fig. 7.—Distribution map for the phloeothripid ? *Haplothrips* sp. T. 2.

numbers, in January (5 samples), February (20), March (17), and early April (2).

T. 3 ? *Haplothrips* sp.

This apparently unrecorded species was taken in small numbers in paddock samples from Te Kauwhata 23.1.57, Maraekakaho 11.2.57, Rotorua 4.3.57, and Maramarua 6.3.57, and in roadside samples from Bulls 30.1.57, Te Mata 4.3.57, and Mangapakeha 25.3.57.

DISCUSSION

Thrips of one species or another were invariably present in the sweep samples. Evidently their size enables them to shelter adequately in even close-grazed pastures. Sweep netting is not considered a particularly efficient method for taking thrips so that considerable populations doubtless exist. Their selective feeding habits, i.e. on growing points and seed heads, may well result in damage that appears to be out of all proportion to the size of the offending individuals.

Only 2 of the 12 species considered here, namely, *Chirothrips pallidicornis* and *Haplothrips niger* have been shown to be of some importance to pasture plants. The role of the remaining species can be determined only by close studies. The numbers encountered in species such as *Anaphothrips obscurus, A. secticornis, Aptinothrips rufus, Chirothrips manicatus,* and *Thrips tabaci,* however, suggest that they too may play some part in pasture productivity.

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THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

VIII. THE ORTHOPTERA AS REVEALED BY SUMMER SWEEP-SAMPLING

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(*Received for publication, 7 August 1959*)

Summary

A sweep-sampling survey during the summer of 1957 revealed 7 species of Orthoptera in the main pasture types occurring in the North Island. The identity, distribution, and abundance of these species are indicated, and their possible economic importance is discussed.

Introduction

During the summer of 1957, a survey of insects associated with pastures in the North Island was carried out. In the first paper of the series (Cumber 1958) techniques, coverage, and relevant sampling station details are given, and the insect complex generally as shown by sweep-sampling is analysed and discussed. Subsequent papers have provided information in respect of the Hemiptera, Coleoptera, Hymenoptera, Psocoptera and Neuroptera, Thysanoptera (Cumber 1959a, b, c, d, e) and Diptera (Cumber and Harrison 1959).

The present account discusses the Orthoptera with regard to the identity of the species, their distribution, numbers encountered, and possible economic importance.

The survey involves 113 paddock and 108 adjacent roadside samples each comprising a single sweep from an area of approximately 8 square yards.

Distribution maps for 3 species are given, a black dot indicating the presence of a species in contrast to a ring where sampling failed to reveal it.

Results

Fam. Gryllidae (Crickets)

Or. 6 *Acheta commodus* (Walk.) (The Common Field Cricket)

Distribution

This species, the common field cricket, which also occurs in Australia, is of widespread occurrence in both North and South Islands, but is more commonly found in northern parts. Tillyard (1926) regards it as having been introduced from Australia. In the present survey, because of its elusive nature and daylight concealment, it was not taken during sweep-netting, but its presence was noted on a number of occasions (Fig. 1) either by direct observation or through its song.

Fig. 1.—Distribution map for the gryllid *Acheta commodus* Or. 6.

Habits and Economic Significance

The general habits of the species are well known, but no detailed studies have been made in New Zealand. From time to time serious local outbreaks have occurred especially in northern areas such as the Hauraki Plains, necessitating resowing of pastures in some instances. Syme (1948) and Banfield and Cottier (1948), give information on the life history and control measures. It appears that eggs are laid in February, March, and April, and that the species overwinters in this stage. Nymphs are to be found from early October onwards. Peak damage occurs in February and March. It is generally agreed that soil conditions which aid severe cracking during dry periods provide optimum opportunity for severe outbreaks. Some types of soil are always prone to cracking and especially so following unusually wet winter conditions. Apparently predation plays an important part in keeping numbers down where refuge is not available in the form of soil cracks.

Pasture composition and grazing management are important factors in resisting the pest, and poison baits are often successful. The pest is essentially a local sporadic one. Since, as indicated by Tillyard (1926), this species has been introduced from Australia, it is possible that parasites which may assist in its control occur in that country.

Or. 3 *Cyrtoxiphus* sp.

This species (16 adults, 45 nymphs) which apparently has not previously been recorded in New Zealand literature, was taken in 4

paddock (6 specimens) and 3 roadside (55) samples from Whangaroa 17.1.57, Gisborne-Tiniroto 12.2.57, Waikohu 13.2.57, Paeroa 5.3.57, Maramarua 6.3.57, and Foxton 4.4.57. Only the last-named sample contained nymphs. Mr E. S. Gourlay who determined this species considers that it may be a fairly recent introduction into New Zealand. It is not important in pastures.

Or. 2 *Lissotrachelus maoricus* (Walk.)

Distribution

This species is known to occur in both North and South Islands. In the present survey it was taken in 34 paddock (154 specimens) and 18 roadside (59) samples, the greatest catch being 8 adults and 8 nymphs from a roadside sample at Warkworth taken on 22.1.57. It is of widespread occurrence in the North Island (Fig. 2).

Habits and Economic Significance

No detailed studies of this New Zealand species have been made. In the present survey, adults and nymphs were taken in January (8 samples), February (12), and March (30), and adults only in early April (2). Lowe (1956) records moderate populations in a turnip field, but does not consider it to be of any real importance.

Fig. 2.—Distribution map for the gryllid *Lissotrachelus maoricus* Or. 2.

Fig. 3.—Distribution map for the tettigoniid *Xiphidium semivittatum* Or. 1.

Fam. Tettigoniidae (Long-horned Grasshoppers)

Or. 1 *Xiphidium semivittatum* Walk.

Distribution

This species, which also occurs in Australia, is present in both the North and South Islands. In the present survey it was taken in 24 paddock (52 specimens) and 45 roadside (335) samples, the greatest catch, 32 nymphs, being in a roadside sample taken from Kai Iwi on 24.1.57. It is widely distributed in the North Island (Fig. 3).

Habits and Economic Significance

The author has observed adults of this species ovipositing in stems of tall fescue at Paiaka on the banks of the Manawatu River. The preponderance of nymphs taken in the early summer suggests that this species overwinters in the egg stage in grass stems. For this reason roadside and waste areas with undisturbed tall grass would appear to favour it, and any damage to pasture could be expected to be adjacent to such areas, or in closed stands. Its importance is difficult to gauge without close studies.

Fam. Acrididae (Short-horned Grasshoppers)

Or. 5 *Phaulacridium marginale* Walk.

Distribution

This New Zealand species is of widespread occurrence in both the North and South Islands. In the present survey it was taken (apparently all adults) in paddock samples from Tolaga Bay 12.2.57 (2 specimens), Opotiki 14.2.57 (1), Mohaka River (Napier-Taupo) 15.2.57 (2), Okere 4.3.57 (1), Kaimai 5.3.57 (1), Pukemiro 5.3.57 (2), and Foxton 4.4.57 (8), and in roadside samples from Uruti 24.1.57 (1), and Foxton 4.4.57 (1).

Habits and Economic Significance

Lowe (1956) records severe damage to turnip, rape, and lucerne near the Rangitata River, and observes that in their migration from adjacent browntop areas these insects consumed a wide variety of weeds indicating their unselective feeding habits. The author has observed similar situations in the North Island where in light sandy soils very considerable populations of this species may occur. As with the common cricket, incidence appears to be closely related to prevailing or preceding weather conditions. They are considered to be only of minor importance in pastures generally.

Or. 7 *Locusta migratoria* L.

Distribution

Uvarov (1928) states that this species, though limited to the Old World, is extremely widely distributed and that, indeed, no other species of Acrididae with such a wide distribution is known. He says it is simpler to state where it does not occur, viz, the colder regions beyond the 60th parallel N. and S., in the dense tropical forests, and in waterless deserts; in mountainous regions it ascends to 15,000 ft. The author has observed it at many localities north of Auckland

throughout the summer months. Adults were seen at Whangaroa on 17.1.57 during sweep-survey operations.

Habits and Economic Significance

The habits of this species have not been studied in New Zealand. The only instance of nymphal occurrence in this country is from Tahunanui, Nelson (Mr E. S. Gourlay, pers. comm.). The numbers encountered give no grounds for suspecting that this species is of importance in pastures.

DISCUSSION

Although moderate numbers of some species of Orthoptera do occur in pastures, there is no evidence, except in the case of the common field cricket, that they are unduly injurious to grazed pastures. The evidence indicates that undisturbed areas of roughage rather than the trampled grazed pastures, are more suited to the build up of orthopterous species, and the relationship of such areas to adjacent pastures and crops will govern the importance of the species. Since orthopterous insects in many other countries often pose very serious pasture problems, New Zealand may perhaps be counted singularly fortunate in the relative paucity of the fauna.

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THE INCIDENCE OF VIRUS X IN SOME NEW ZEALAND POTATO VARIETIES

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(*Received for publication, 3 August 1959*)

Summary

The incidence of potato virus X in several varieties has been determined by the field agglutination test. The varieties Aucklander Short Top, Dakota Red, Arran Banner, Arran Consul, and Arran Pilot were one hundred per cent infected, while Chippewa and Sebago had a high degree of infection. No X-infected plants of the New Zealand variety Ilam Hardy could be found, and it appears to be field-immune. Two recently released varieties, Constellation and Southern Cross, and two introduced varieties, Ulster Beacon and Ulster Supreme, had a low degree of infection. The incidence and spread of the virus are discussed and the means of control outlined.

Introduction

Certification schemes for seed potatoes are based on visual inspection and roguing of the growing crop, hence virus diseases that show obvious symptoms, such as leaf roll and severe mosaic, can be eradicated. On the other hand virus diseases that produce few observable symptoms will tend to increase and, ultimately, varieties may become completely infected. Potato virus X, usually known in New Zealand as mild mosaic, is a good example of such a disease. It exists in several different strains, of which some produce distinct interveinal mottling but most do not show any symptoms on potato plants. Potato varieties vary greatly in their reaction to the different strains. The masked strains can reduce yield by 10% to 20% (Bald 1944; Schultz and Bonde 1944), while severe strains can cause greater reductions in yield (Klinkowski 1951). Of more importance is the interaction of virus X with other potato viruses such as virus A (crinkle) and virus Y (rugose mosaic). In combination these cause severe reductions in yield, and varieties wholly infected with virus X degenerate rapidly in poor seed areas where A and Y are readily spread by aphids. The New Zealand certification scheme for seed potatoes has been successful in reducing the incidence of the aphid-transmitted potato virus diseases. However, masked viruses have spread unchecked, so that they now probably reduce yields more than do all the other potato viruses.

In 1949, Procter (1952) tested most New Zealand varieties for virus X. Since then new varieties have been introduced and there have been changes in the importance of older varieties. These changes are shown in Table 1, which gives the acreages grown for certified seed, in five recent seasons. The figures are from the lists of Provisionally Certified Growers.

The results of field tests in 1958 and 1959 for virus X in several potato varieties are presented in this paper.

TABLE 1. Area in Acres of Certified Seed Potatoes Grown in New Zealand

Variety	1948-49	1951-52	1956-57	1957-58	1958-59
Arran Banner	696	355	318	328	349
Arran Chief	902	532	400	332	249
Arran Consul	—	4	10	12	22
Arran Pilot	—	12	2	2	—
Aucklander Short Top	1913	1459	1746	1651	1619
Aucklander Tall Top	194	196	176	76	60
Chippewa	25	117	210	332	601
Cliff's Kidney	43	28	33	49	66
Constellation	—	—	14	34	46
Craig's Snow White	—	—	2	4	2
Dakota Red	582	454	852	738	678
Epicure	100	94	100	90	125
Glen Ilam	27	79	135	159	158
Ilam Hardy	—	6	227	598	735
Inverness Favourite	152	66	100	86	114
Iron Duke	40	72	26	17	12
Jersey Bennes	32	21	10	18	27
Katahdin	50	74	132	168	281
King Edward	197	106	136	214	199
Red King Edward	—	—	8	15	18
Sebago	133	130	670	922	1239
Ulster Supreme	—	—	—	3	6
Total	5086	3805	5305	5848	6606

Methods

Virus X rarely produces symptoms on potato plants and so it is necessary to test for it either by inoculation to indicator plants or by the use of serological methods. The field agglutination technique of Bradley (1952) based on plant virus serology, was chosen. The unabsorbed virus-X antiserum was imported from Holland, and normal horse serum was obtained locally. Both were used at a dilution of 1 : 40. The test consists of placing on a slide two drops of crude sap, extracted from the potato leaves with a specially made hand press, and adding a drop of normal serum to one and a drop of antiserum to the other. Each drop is then mixed with a toothpick. Only if the sap is from an X-infected plant will there be an aggregation of the chloroplasts in the antiserum and there should be no reaction in the normal serum. In 1958 the tests were done on glass slides, one per test as suggested by Bradley (1952). However, in 1959 it was found much more convenient to do them on 10 in. by 4 in. sheets of $\frac{1}{8}$ in. Perspex, five tests per sheet. There was little tendency for the drops to run together. Light reflected from a mirror below the slide was an aid in determining agglutination.

One of the conditions of the New Zealand certification scheme for seed potatoes is that a 150-tuber sample of each line entered for

certification be grown in trial plots at Timaru or Dunedin. This enables comparisons between lines to be made under uniform and as nearly ideal conditions as possible. In 1958, the tests were done at Timaru, the first ten plants in a row being tested. In 1959, samples collected from plants chosen at random within the lines grown at Timaru were tested in the laboratory. All lines tested were of mother-seed origin and the tested lines were chosen to cover a number of different localities in New Zealand. At the time of testing (early January) the plants were beginning to flower.

Tests were made also on several commercial crops, although in only one case was a test done on a line which had been tested in the trial plots. Eighty consecutive plants of this line were tested but all other crop tests were done on plants chosen at random.

RESULTS

The incidence of potato virus X in some New Zealand varieties is presented in Table 2.

In the varieties Chippewa and Sebago there was some variation from line to line in the incidence of virus X, but this was not signi-

TABLE 2. INCIDENCE OF VIRUS X IN SOME NEW ZEALAND POTATO VARIETIES

Variety	Total No. of Lines in Certification Plots	Plants Tested	Percentage Infected
1958 Tests—			
Chippewa	87	100 (10 lines of 10)	75
Constellation	4	20 (2 lines of 10)	0
Ilam Hardy	117	100 (10 lines of 10)	0
Sebago	234	100 (10 lines of 10)	79
Ulster Supreme	3	20 (2 lines of 10)	25*
1958 Crop Tests—			
Sebago, Tuatapere		80 (1 line)	64
Arran Pilot, Lincoln		20 (1 line)	100
1959 Tests—			
Arran Banner	85	40 (2 lines of 20)	100
Arran Consul	3	20 (1 line of 20)	100
Constellation	8	20 (1 line of 20)	5
Southern Cross	1	20 (1 line of 20)	25
Ulster Beacon	1	20 (1 line of 20)	20
Ulster Supreme	3	20 (2 lines of 10)	0
1959 Crop Tests—			
Arran Banner, Darfield		20 (1 line)	100
Sebago, Annat		50 (1 line)	98
Sebago, Lincoln		50 (1 line)	94
Ilam Hardy, Lincoln		100 (1 line)	0

* One line was X-free.

ficant. A line of Sebago with 40% infection in the trial plots was re-tested in the field where it showed 64% infection.

The main potato variety in New Zealand, Aucklander Short Top, was not tested in this investigation. However, in 1954 Miss C. M. Smart, formerly of this Division, tested four lines of this variety for X and Y; sixty plants being inoculated to *Datura stramonium* and *Nicotiana tabacum* var. White Burley. Although all were infected with virus Y, some appeared free from X because of the absence of symptoms on the *Datura* plants. Tubers from plants apparently free from X were re-grown and re-tested by serology, with the indicator plants already mentioned and also with *Gomphrena globosa*. All of the potato plants gave positive precipitin tests with virus-X antiserum, but *Gomphrena globosa* was the only indicator plant that reacted with symptoms. Thomson (1956) has since shown that there are very mild strains of virus X in this variety and that they produce no symptoms on most test plants.

Mr A. D. Thomson, formerly of this Division, also carried out many tests on Aucklander Short Top and found none free from virus X.

Dakota Red is another important variety not tested in the present work. No X-free plants could be found in tests by C. M. Smart and A. D. Thomson on several hundred plants.

Discussion

Kristensen (1955) considered that the field agglutination test for virus infection is very reliable and a useful method for field surveys, although Bercks (1956) obtained a reliability of only 80%. In this study and in other work at this Division on potato virus X, the technique has always given satisfactory results. It has the advantage over tests in which indicator plants are used in that immediate results are obtained. At the time of testing, current-season spread of virus X was probably not detectable by the field agglutination test and so has not affected the overall results.

That most of the important potato varieties grown in New Zealand are heavily infected with virus X has been shown by the results reported here and those of Procter (1952). Tests in 1949 (Procter 1952) on the varieties Chippewa (one line) and Sebago (five lines) showed 60% and 78% infection with virus X respectively. It appears that there has been little change over the last ten years in the incidence of virus X in these two varieties (assuming that the lines tested were representative). Since virus X is spread mainly by contact of foliage one would expect that they should have become entirely infected. However, Todd (1958) has also shown that there is great variation in the incidence of this virus in commercial seed and that some varieties do not appear to become one hundred per cent infected. Bawden *et al.* (1948) and Roberts (1948) have shown that severe strains of virus X reach a higher concentration in plants than mild strains and are spread more rapidly. However, the severe strains usually produce obvious symptoms and therefore infected plants can be rogued. As mild strains produce no symptoms they have a survival advantage over

the severe ones, but apparently they spread so slowly that complete infection is seldom attained. Infection of plants and progeny is often only partial and this is probably also important in maintaining some virus-free tubers.

Potato virus X, being a relatively stable virus, is readily transmitted mechanically. It can be spread by contact between leaves, roots, and sprouts of diseased and healthy plants. It can also be carried from plant to plant on the hands and clothing of people walking through crops, on cultivating implements, and on the fur of animals (Todd 1958). These latter means of transmission are more effective in wet weather and are not only important in increasing the incidence within a crop but are more important in introducing the virus from outside sources into virus-free crops. Susceptibility is greater in young than in mature plants.

Control of virus X may be attempted in two ways. The first method is by the selection of X-free plants, their multiplication in isolation, and finally their use to replace commercial stocks. The conditions for maintaining virus-X-free stocks have been described by McIntosh (1958). It is important that X-free crops should be maintained on land free from other potatoes. Doncaster and Gregory (1948) have shown that volunteers are an ideal source of infection, and they may survive for several years. The conditions for growing X-free seed are being applied in New Zealand as far as possible in the maintenance of an X-free line of Arran Chief. Information on the incidence of virus X in this variety will be presented in another paper. An X-free line of Sebago has also been selected and is being increased.

The second method of control is the production of field-immune (hypersensitive) or immune varieties. Field immunity is present in the varieties King Edward, Epicure, and Jersey Bennes (International Kidney). However, there are strains of virus X to which they are susceptible and tolerant. Immunity derived from the U.S.D.A. seedling 41956 is more desirable since it has been shown to be at present immune to all strains.

It is clear that unless new varieties are either field-immune or immune, they will, unless grown in isolation, soon become infected with virus X. The New Zealand varieties Constellation* and Southern Cross which have been recently released showed some infection in these tests.

Two other New Zealand varieties, released several years ago, are Glen Ilam and Ilam Hardy. The first has been found to be 60%

* Constellation, bred by Mr C. M. Williams, Kaiapoi, is from the cross Aucklander Short Top × Chippewa. Southern Cross, bred by Mr T. A. Penn, Christchurch, has Katahdin as one of its parents. Glen Ilam (Catriona × Chippewa) and Ilam Hardy (Arran Pilot × Chippewa), were both bred by Mr R. G. Robinson, Christchurch.

Chippewa and Katahdin as grown in New Zealand appear to be identical. Both were probably introduced but Chippewa may have subsequently been replaced by Katahdin. It is likely that Katahdin was the pollen parent of Constellation, Glen Ilam, and Ilam Hardy.

infected with virus X (Procter 1952) while no infected plants have been found of the variety Ilam Hardy. It is reasonable to suggest that it is field-immune to virus X and this is being tested.

The Crop Research Division of the Department of Scientific and Industrial Research is selecting virus-X-immune varieties. This work was begun in November 1951 when X-immune parental material (119 series) derived from U.S.D.A. 41956 was received from Dr E. M. Hutton of Canberra, Australia. Many crosses have since been made with commercial varieties and blight-resistant material. Advanced hybrids are now being tested for their suitability for New Zealand conditions.

Acknowledgements

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SOME EFFECTS OF THYROXINE ON LIVE WEIGHT, METABOLISM AND WOOL GROWTH OF ROMNEY EWES

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Summary

An experiment was carried out to study the effects of L-thyroxine on the live weight, metabolism, and wool growth of 12 Romney ewes. The thyroxine was administered by implantation and by injection.

The injection of 5 mg thyroxine/day increased the oxygen consumption, heart rates, and respiration rates of the treated ewes. No effects were observed on rectal temperatures of the treated animals, but their live weights were reduced.

When 1 mg thyroxine/day was injected, the oxygen consumptions, heart rates and respiration rates of the treated animals were increased. No effects could be demonstrated on rectal temperatures and live weights.

A dose of 0.75 mg thyroxine/day was sufficient to produce a measurable increase in heart rate, but no effect was observed on live weight.

Thyroxine injection produced a highly significant increase in wool growth.

No effects of thyroxine implantation were observed in this experiment. Possible reasons for the differences in results following the use of different methods of administration of thyroxine are discussed.

Introduction

Blaxter *et al.* (1949) have reviewed the literature on the use of thyroid-active substances which have been investigated in attempts to increase meat, milk, and egg production. More recently, evidence has been produced to show that iodinated casein (Maqsood 1950, 1955) or thyroxine (Hart 1954, 1955, 1957, 1958; Labban 1957; Ferguson 1958; Ross and Lewis 1958; Coop and Clark 1958) administered to normal sheep causes a stimulation of wool growth and affects reproduction and lactation. The workers using thyroxine have found positive responses following injection, oral administration, or implantation of tablets subcutaneously.

Measurement of the degree of hyperthyroidism produced has been lacking in most experiments involving either oral administration or implantation. The rate of uptake of thyroxine from implanted tablets has likewise not been measured.

The present experiment was concerned with the effects of implanted and injected thyroxine on wool growth, live weight, heart rate,

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respiration rate, rectal temperature, oxygen consumption, food intake, and the rate of uptake of thyroxine from the implanted tablets.

MATERIALS AND METHODS

On 12 April 1958, 12 mature Romney ewes ranging from 120 to 112 lb live weight were randomised into two groups of six sheep. On 14 April 1958, those in one group were surgically fitted with modified field-pattern tracheotomy tubes of the type developed by Cresswell (1958) for energy metabolism studies using an oxygen spirometer.

The non-tracheotomised ewes were randomised to two groups of three animals, and those in one group were implanted with 90 mg thyroxine*/ewe; those in the other group were not implanted (Table 1).

TABLE 1. THYROXINE TREATMENTS APPLIED TO THE EWES

	Ewe No.	Implantation 1958 24 Apr	Injections			
			3, 4, 5, and 6 June	17-24 June incl.	2-10 July incl.	11-15 July incl.
Tracheotomised	28	90 mg	....	....		
	25	90 mg				
	23	90 mg	5 mg/day	1 mg/day		
	21	....	5 mg/day	1 mg/day		
	17					
	583*					
Non-tracheotomised	580	....	5 mg/day	1 mg/day	0.5 mg/day	0.75 mg/day
	589	....	....	....	....	....
	18	....	....	....	0.5 mg/day	0.75 mg/day
	9	90 mg	5 mg/day	1 mg/day	0.5 mg/day	0.75 mg/day
	584	90 mg				
	5	90 mg				

The tracheotomised ewes were slaughtered 26 June.

* Died 26 May.

Three of the six tracheotomised ewes were selected for implantation on the basis of two criteria. First, it was found by Cresswell (1958) that animals which submit to handling and pen feeding are needed if reliable recordings of oxygen consumption are to be obtained. Secondly, animals likely to survive the duration of an experimental period were needed to enable the treatment effects to be assessed. The results of implantation and subsequent treatments could be judged by measurements taken on the same animal before and after the treatment effects were observed. Details of the tracheotomised ewes which were implanted with 90 mg thyroxine/ewe are shown in Table 1.

* Throughout these experiments purified L-thyroxine was used.

The remaining three tracheotomised ewes were considered unsuitable for implantation for the following reasons: Ewe 583 had been losing weight steadily since the tracheotomy operation and had lost her appetite; ewe 17 was very excitable and did not appear to be respiring normally; ewe 21 refused to eat concentrates.

Thyroxine tablets were implanted subcutaneously on 24 April 1958 with a short-needled implanting gun at a point behind the shoulder about two inches to the left of the centre line of the back. At the same time wool samples were taken from all ewes on a standard side position just behind the last rib. An area of about 60 sq. cm. was clipped from each of the left and right sides using a No. 0000 Oster clipper. Further samples were taken from the same area on 3 June and again on 24 June. The wool samples were weighed, then scoured and re-weighed after conditioning at 64% r.h. and 68°F.

All animals were individually penned in a shed and fed a basic ration of 1 lb of crushed oats and $\frac{1}{4}$ lb of kibbled peas per day plus hay *ad lib.* In order to allow an estimate of the food consumption per ewe to be obtained, the ration was weighed and the uneaten portion weighed back the following day. The ewes were bedded on a litter of sawdust.

The oxygen consumptions and respiration rates (mean rates over 3 one-minute periods per ewe) of the six tracheotomised ewes were measured between 7 and 9 a.m. on a McKesson basal metabolic rate (b.m.r.) machine (spirometer) which automatically records oxygen consumption and respiration rate (Cresswell 1957; Webster and Cresswell 1957; Cresswell 1958). In this connection, Brody (1945, p. 311) considered that for ruminants the rate of oxygen consumption is the best indirect measure of heat production. The rationale for this procedure and the difficulties in trying to take respiratory quotient into consideration in metabolic studies with the ruminant are discussed by Brody.

On the mornings that oxygen consumptions were recorded, they were the first measurement taken. All ewes were weighed and then fed daily before midday.

Measurements of heart rate, respiration rate, and rectal temperature were made between 2 and 5 p.m. The ewes were trained to stand in a corner of their pen and their heart rate was measured with the aid of a stethoscope. Repeated handling made the animals very docile, and some of the animals would ruminate while heart rate was being recorded. For graphical purposes, the heart rates of each ewe were averaged from 12 May to 3 June inclusive, and all heart rates of each ewe have been expressed as deviations from their individual mean over this selected portion of the implantation period.* These deviations were then grouped and averaged to allow different comparisons to be made (Figs. 2 and 4).

* The period 12 May to 3 June was a selected portion of the implantation period (24 April to 3 June). Observations from the implantation period taken before 12 May were discarded as the animals were being trained and some high recordings and large fluctuations were noted initially.

As no effects of thyroxine implantation could be detected by 2 June (39 days after implantation) it was decided to test the thyroxine by injection. An alkaline thyroxine solution containing 5 mg thyroxine/cm^3 was made up from the same stock of implant tablets as had been previously used. Table 1 gives details of the four animals which were injected and the duration of the treatment. Control injections of alkaline distilled water were given to two other animals. Injections were always given in the late afternoon, after all measurements had been taken on the ewes.

From 17 to 24 June inclusive, the same four treated ewes were injected with 1 mg thyroxine/day (Table 1). On 26 June (63 days after implantation) the tracheotomised ewes were slaughtered and the residual thyroxine implant tablets were saved from ewes 28, 25, and 23. These residual implants were analysed for iodine using the method of Kendall (1920), and similar analyses were carried out on unused 30 mg thyroxine tablets. An estimate of the amount of thyroxine absorbed from the implanted tablets was then obtained.

After the heart rates of the remaining two thyroxine-injected ewes (580 and 9) in the non-tracheotomised group had dropped to normal, these ewes, plus a further ewe (18) were given lower levels of thyroxine injection (Table 1). The remaining three ewes were given control injections of alkaline distilled water. From 27 June the animals were fed only on hay. The experiment was concluded on 28 July 1958.

Included for comparative purposes are the results of an earlier experiment, in which the effects of the injection of 5 mg thyroxine/day on a 4-year-old tracheotomised Romney ewe were measured (Fig. 3). The experiment took place over the period 28 January to 8 March 1957 (summer-autumn). The thyroxine solution used in this earlier experiment was prepared from implant tablets which caused physiological responses during a 28-day implantation experiment (Kirton and Barton 1958).

Results

Analyses of Residual Implants

The thyroxine tablets used in this experiment were checked for purity against a higher-grade sample of thyroxine by an analysis for iodine; they had 96% of the iodine content of the higher-grade sample.

The analyses of the residual implants from three ewes (23, 28, and 25) showed that 82.6%, 89.8%, and 68.6% of the 90 mg of thyroxine originally implanted, remained after 63 days. The figure of 68.6% is an underestimate as an appreciable loss of iodine occurred from implant 25 during the alkaline fusion, because the material suddenly erupted. This almost certainly accounts for the lower iodine recovery from this implant. As even with the other two implants some material may have been lost during collection and analysis, all figures are likely to underestimate the amount of thyroxine remaining. Also, the fact that the thyroxine tablets removed from the three ewes at slaughter appeared to retain their original size and shape is further evidence suggesting that little thyroxine was absorbed.

In the light of the above evidence, if the figure of 82% or more of the 90 mg of thyroxine originally present is assumed to remain, then 16 mg or less thyroxine may have been absorbed by the implanted ewes over the 63 days of implantation. If the assumption is made that the thyroxine was absorbed at an even rate, then these results suggest that the implanted ewes were absorbing less than 0.25 mg thyroxine per day.

Effects of Thyroxine Implantation, 24 April to 3 June (40 days)

All ewes, except 583, gained in weight over this period. This ewe refused to eat the hay and concentrates after the tracheotomy

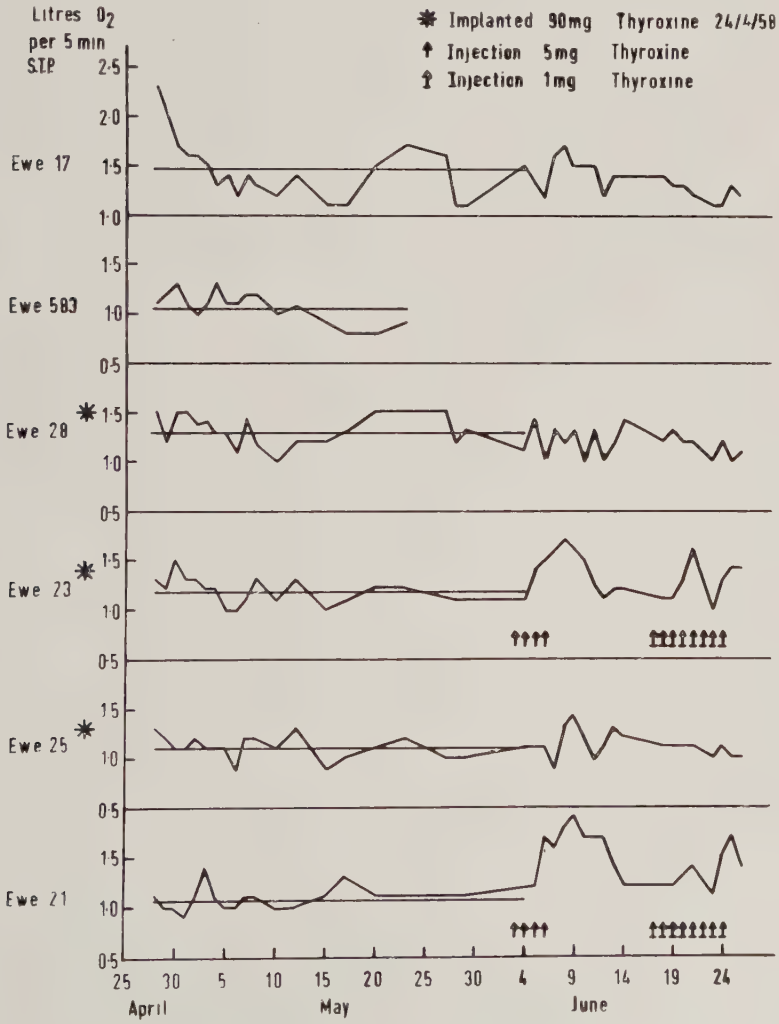

Fig. 1.—Oxygen consumptions expressed as deviations from the mean oxygen consumption over the implantation period for each ewe.

operation. She was put on to pasture daily and seemed to graze normally, but died on 26 May after losing a total of 25 lb live weight.

No effects of implantation could be detected on the oxygen consumption of the tracheotomised ewes (Fig. 1). All readings in Fig. 1 have been expressed as deviations from the mean recordings to 3 June. Because of a leak in the basal metabolism machine, readings of oxygen consumption taken between 24 and 27 April had to be discarded. Ewe 17, a very excitable animal which had not been implanted, produced abnormally high readings when compared with the other tracheotomised ewes during the first few days of the experiment. This ewe was observed to spend long periods of the day puffing (as though from violent exercise) but was considered by a veterinarian to be normal.

TABLE 2. Respiration Rate/min of the Tracheotomised Ewes as Measured on the B.M.R. Machine. Means and Standard Deviations (S.D.)

Dates	25/4/58-3/6/58			4/6/58-26/6/58			25/4/58-26/6/58
Ewe No.	n	Mean	S.D.	n	Mean	S.D.	Range
28*	23	45	9	19	45	8	20–63
25*	23	22	3	19	29	5	19–42
23*	21	45	10	18	64**	14	31–98
17	23	56	11	18	57	7	39–77
583†	20	23	2				19–27
21	23	43	11	19	59**	13	22–83

* Implanted.
** Injected.
† Died 26 May
n = Number of observations on which the mean is based.

As shown in Table 2, there were no differences between the respiration rates of the implanted tracheotomised ewes and those that had not been implanted. Similarly, no effects of implantation of thyroxine were observed on respiration rates when these were measured independently of the b.m.r. machine.

The mean heart rates of all animals (excluding 583 which died) are recorded in Table 3. Readings taken before 12 May were discarded for the reasons given earlier. During this first period the mean heart rates of the six implanted ewes were 80 beats/min. The mean heart rates of the ewes which were not implanted also averaged 80 beats/min.

No differences attributable to thyroxine implantation were detected in rectal temperature or in food consumption between the implanted and the non-implanted groups of ewes.

TABLE 3. MEAN HEART RATE PER MINUTE AND STANDARD DEVIATIONS (S.D.) FOR SELECTED PERIODS OF THE EXPERIMENT

	Implantation 12 May-3 June incl.		Injection (5 mg Txn) 5–14 June incl.			Injection (1 mg Txn) 19-25 June incl.			Control 27 June-2 July incl.			Injection 9-20 July incl.			Control Period 4 23-28 July incl.		
Ewe No.	Mean (M_1)	S.D.	Mean (M_2)	S.D.	M_2-M_1	Mean (M_3)	S.D.	M_3-M_1	Mean (M_4)	S.D.	M_4-M_1	Mean (M_5)	S.D.	M_5-M_1	Mean (M_6)	S.D.	M_6-M_1
28*	79	8	75	7	— 4	75	7	— 4									
25*	92	12	82	7	—10	80	3	—12									
23*	78	5	91**	4	13	90**	8	12									
21	66	3	92**	8	26	87**	7	21									
17	93	4	96	4	3	93	6	0									
580	85	9	103**	7	18	93**	7	8	69	5	—16	76**	4	— 9	64	2	—21
18	81	10	76	6	— 5	67	8	—14	57	4	—24	71**	6	—10	56	2	—25
589	74	8	68	4	— 6	63	4	—11	56	4	—18	55	4	—19	50	3	—24
584*	70	7	65	3	— 5	67	5	— 3	54	3	—16	54	3	—16	51	4	—19
9*	84	6	109**	10	25	102**	10	18	68	4	—16	79**	7	— 5	67	2	—17
5*	74	9	72	4	— 2	69	4	— 5	59	2	—15	63	4	—11	63	3	—11
Mean	80								60		—18				59		—20

* Implanted 90 mg thyroxine 24/4/58.
** Injected.

Thyroxine implantation did not affect wool growth (Table 4). The lower weight of wool produced by the non-implanted tracheotomised ewes can be explained by the same reasons which resulted in the selection of these two ewes for their particular group.

TABLE 4. The Mean Wool Weights (units = g × 10) of Implanted and Control Sheep of the Tracheotomised and Non-tracheomotised Groups

Dates	Non-tracheotomised		Tracheotomised	
	Implanted	Not Implanted	Implanted	Not Implanted (2 ewes)
From shearing to 24 April	3324	3425	3107	3045
24 April-3 June	575	548	495	291
Live-weight gains (lb)	9	6	11	5

Effects of Four Daily Injections of 5 mg Thyroxine/Day, 3 to 16 June

Loss of live weight started in all injected ewes the day after the first injection and continued until the day after the last injection. By this time the injected ewes had on average lost 7 lb live weight and the remaining ewes had lost 1 lb live weight. The resulting live-weight difference between the injected and the non-injected groups of ewes could still be detected two to three weeks later.

Fig. 1 shows that the oxygen consumptions of the two injected ewes in the tracheotomised group started to rise 2-3 days after the first injection, and rose to values of up to 140% and 180% of the mean values respectively over the previous period. These increases persisted for several days after the injections were stopped. The results are similar to those previously observed at this College (Fig. 3) on a Romney ewe which had been used in energy metabolism experiments.

The heart rates of the injected animals started to rise on the second day after injection (Fig. 2). The increase in heart rate of the injected animals was of the order of 25 beats/min as compared with the non-injected animals (Table 3).

Effects of Eight Daily Injections of 1 mg Thyroxine/Day, 17 to 26 June

No effects on live weight of thyroxine injection at this level could be detected.

The oxygen consumption of the injected tracheotomised ewes started to rise on the third day after the injections began (Fig. 1) and rose to values of up to 130% and 160% of the mean values over the implantation period. Recordings from the injected ewes were consistently higher (except for 23 June) than their mean value over the implantation period, whereas the remaining three tracheotomised ewes had lower oxygen consumptions than for the implantation period.

Difficulty with the sticking of valves on the b.m.r. machine was experienced over the latter part of the experiment. This is thought

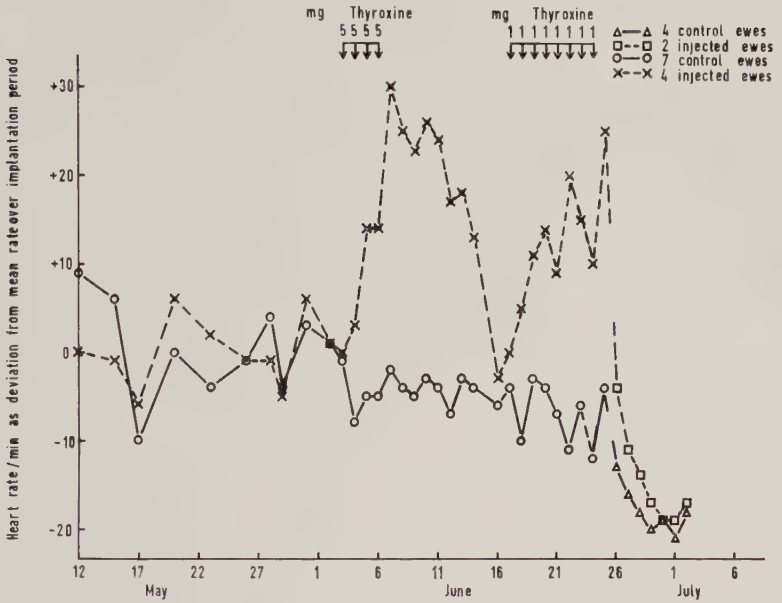

Fig. 2.—Effects of injection of 5 mg and 1 mg of thyroxine per day on heart rate.

to be the explanation for the low readings recorded on 23 June, although the evidence on this point was not good enough to warrant the discarding of these data. However, independent evidence to support this suggestion can be seen in Fig. 2, which shows that heart rate remained high for the injected ewes on this day.

The heart rates of the injected ewes started to rise on the second day after injection commenced (Fig. 2) and it can be seen from Table 3 that the heart rates of the injected animals were increased by 22 beats/min when compared with the non-injected ewes.

Respiration Rate, Rectal Temperatures, Food Intake, and Wool Growth, 3 to 26 June

The respiration rates of the injected tracheotomised ewes were slightly elevated by both the 5 mg and the 1 mg levels of injection, as compared with the readings in the previous implantation period (Table 2). No differences could be detected between the effects of the two levels of injection. When the respiration rates of all ewes were determined by counting independently of the b.m.r. machine, a similar small increase was noted. The observed increase was small, however, when compared with the spectacular increase in the respiration rate of a ewe injected with 5 mg thyroxine in summer (Fig. 3).

The mean rectal temperature of the four injected ewes was 102.4°F over the periods that the animals were hyperthyroid and 102.5°F over the previous implantation period. The corresponding figures for the seven non-injected ewes were 101.8°F and 102.3°F respectively. These figures indicate no effects due to thyroxine injection.

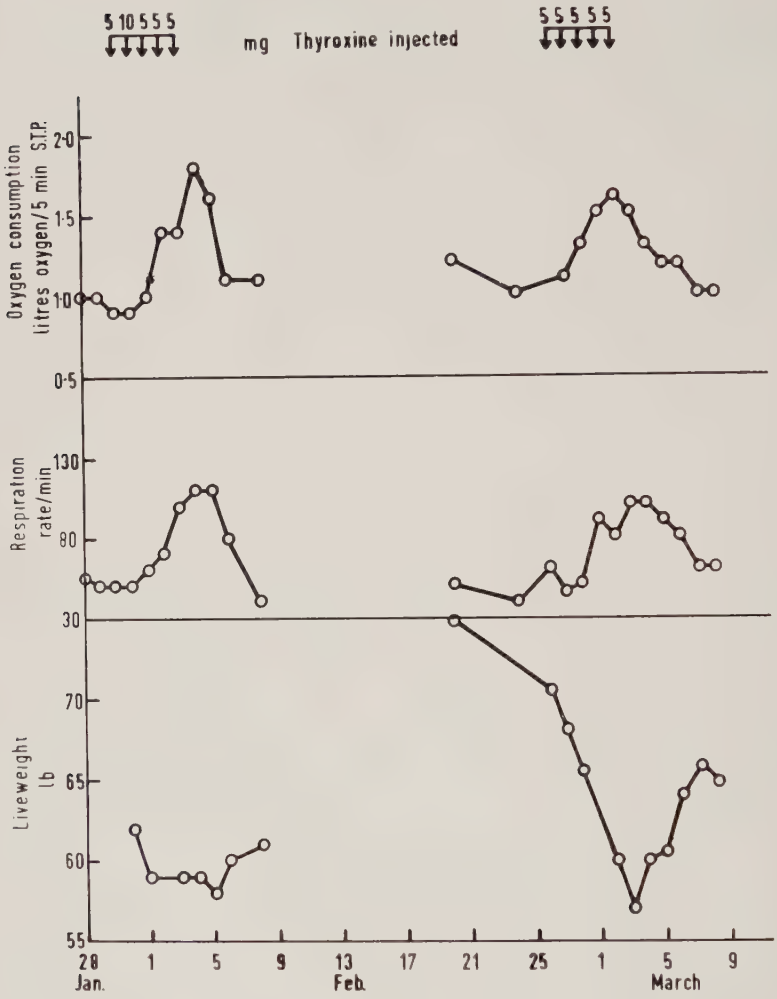

Fig. 3. The effects of thyroxine injection on the oxygen consumption, respiration rate, and live weight of one Romney ewe, 1957.

No differences attributable to thyroxine injection were found in the food consumption of the ewes. However, only large differences would have been significant when judged by the experimental methods used.

As no effects of implantation had been demonstrated on wool growth or on any of the other variables measured, the growth over the period 24 April to 3 June was used as the independent variable for an analysis of covariance of wool growth from 3-24 June. Such an analysis allows for variation arising from normal differences in rate of wool growth amongst the sheep and also for any variations in the areas from which the samples were taken. The analysis of the data

(Table 5) shows a highly significant difference in wool growth between the groups. The injected sheep produced 70% and the non-injected sheep 51% of their wool growth over the implantation period (24 April to 3 June). It was concluded that the thyroxine injections caused a marked increase in wool growth.

TABLE 5. THE ANALYSIS OF COVARIANCE OF WOOL GROWTH (units = g × 10) DURING THE INJECTION EXPERIMENT FOR 11 EWES

Source	d.f.	Residual S.S.	Residual Mean Sq.	F
Total	9	25,504.74		
Groups	1	17,301.50	17,301.50	16.87 $P < 0.01$
Sheep	8	8,203.25	1,025.41	

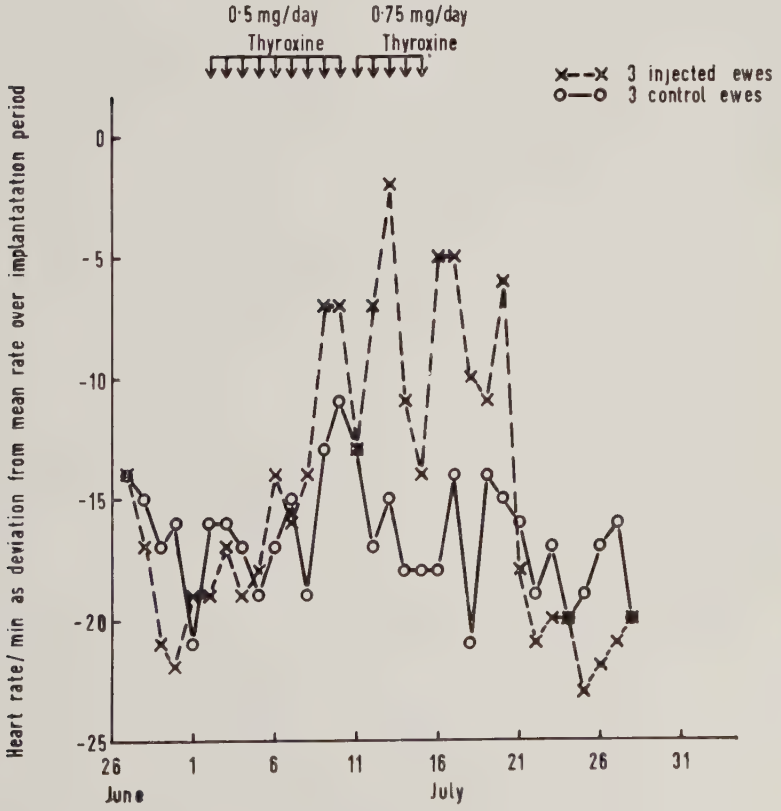

Fig. 4.—Effects of injection of 0.5 and 0.75 mg of thyroxine per day on heart rate.

Effects of 0.5 and 0.75 mg Thyroxine Injection, 27 June to 28 July

Fig. 4 shows that 0.5 mg thyroxine did not produce any detectable effect on the heart rate of the injected ewes, but 0.75 mg appears to have elevated it. By comparing the heart rates from 9-20 July with the heart rates immediately before and after this period (Table 3) it can be seen that the thyroxine injection raised the heart rate by 10 beats/min.

These injections had no effect on live weight.

Discussion

No effects were observed on any of the variables measured over the first 40 days of the experiment, from the implantation in late April of 90 mg thyroxine in Romney ewes. An explanation for these results was found from the chemical analyses of the residual implants from 3 tracheotomised ewes. These analyses indicated that most of the thyroxine remained (82% or more) following 63 days of implantation. The physical appearance of the removed tablets also supported this evidence. These results may be contrasted with those of an earlier experiment lasting only 28 days, at the end of which the residual thyroxine tablets were in the form of a pulp and chemical analyses indicated that 56% or more of the thyroxine remained (Kirton and Barton 1958, p. 277). Possible differences between batches of thyroxine implant tablets have been commented on by Hart (1958).

The results of the chemical analyses suggest that the implanted ewes in the present experiment were absorbing 0.25 mg or less thyoxine/day from their implants. As 0.75 mg or more thyroxine was necessary to increase the heart rate of the injected ewes in the present experiment, and as heart rate was the most sensitive indicator of thyroxine therapy studied, it is not surprising to note that no effects of thyroxine implantation were observed throughout these experiments. Furthermore, Henneman *et al.* (1955) have published evidence which suggests that Shropshire and Hampshire ewes may normally secrete up to 0.35 mg L-thyroxine/day.

When the thyroxine tablets were dissolved and injected at a known rate in the present experiments, similar effects to those observed after injection in previous experiments were noted. For example, 5 mg thyroxine/day produced live-weight losses similar to those observed with thyroxine dissolved from an earlier batch of implant tablets (cf. Fig. 3, also Kirton and Barton 1958). In the same way, the rise in oxygen consumption caused by this level of thyroxine injection was of the same order as observed previously (cf. Figs. 1 and 3). These results show that the thyroxine from the different batches of implant tablets had similar biological activity but, as the earlier evidence suggests, the thyroxine from some batches of tablets was absorbed more easily than from others.

The rise in respiration rate was, however, much less in the present experiment (Table 2) than in an earlier experiment (Fig. 3); in both cases respiration rates were measured on the b.m.r. machine. This difference between the two results may be explained by the difference

in environmental temperature, the mean monthly temperature in June 1958 being 48°F and in February 1957 being 66°F. Blaxter (1948) states: "It has been shown that hyperthyroidism in the ruminant does not specifically affect respiration rate, other than through increasing heat production". Because of the comparatively low ambient temperatures during the present experiment, it is likely that the hyperthyroid animals experienced little difficulty in disposing of the extra heat produced, and so the rise in respiration rate was small. It was noted that the respiration rate, when measured both before and after a recording was taken, was higher for a sheep in its pen than when it was connected to the b.m.r. machine.

A daily dose of 5 mg thyroxine markedly increased the heart rates of all treated animals.

It was of interest to note, however, that the rectal temperatures of the injected animals were not affected by thyroxine treatment. The mean of all recordings taken during the experiment was 102.2°F and the range was 100.8–103.6°F which almost coincide with the figures regarded by Dukes (1947) as normal for sheep. It is probable that the comparatively low mean environmental temperature mentioned earlier explains the lack of effect of thyroxine on rectal temperatures in the present experiment. Higher environmental temperatures may explain the increase in rectal temperatures following thyroxine treatment reported by Ferguson (1958) from Australia. The short duration of the treatment period in the present experiment could also be a contributing factor.

The results of Lambourne working at Armidale (pers. comm.) have shown that 7 mg L-thyroxine injected once weekly produces similar effects to a 90-mg thyroxine implant under Australian conditions. As many New Zealand implantation experiments have used the 90 mg implant level, 1 mg thyroxine/day was injected and the results were observed. This treatment produced definite evidence of hyperthyroidism, as indicated by an increased oxygen consumption, raised heart rate, and a slight increase in respiration rate. No effects could be seen in terms of live-weight change or rectal temperatures. This suggests that live-weight loss may not be the best measure for the biological activity of thyroxine, although it is possible that the 8-day duration of the injection treatment was not long enough to produce a live-weight loss.

The injection of thyroxine resulted in an increase in wool growth over the same periods that increases in the metabolic rates of the injected animals were obtained. Similar results have been observed by Ferguson (1958) who suggested that an increase in wool growth is a result of an effect of thyroxine on metabolism. However, Myers and Ross (1959) have evidence from a thyroxine implantation trial of an increase in metabolic rate (as measured by heart rate) which was not accompanied by an increase in wool growth. In their experiment the rate at which the animals were absorbing the thyroxine was not measured.

Coop and Clark (1958) have compared the gains in fleece weight resulting from implantation with 60 mg or more of thyroxine at

different times of the year. Gains in actual fleece weight were found from implantation in April, June, and September but not in December, when 4 out of 11 implanted ewes died. The authors, however, also measured wool growth by clipping a delineated area at regular intervals and base their conclusions on "estimated greasy-fleece weight calculated from the clean scoured wool of the clippings and an assumption (which is known to be approximately correct) of 10 lb as an average annual greasy fleece weight". The rationale of such an estimation is not made clear but the authors conclude from greasy-fleece weights estimated in the above manner that thyroxine is effective only in late summer and autumn. Thus their actual fleece-weight data agree with the present result of a wool-growth increase following thyroxine administration in June, but their conclusions based on calculated gain in fleece weight differ. Because of lack of information on the variability of the sheep used and allowance for possible effects of age and number of lambs, it is not possible to assess these results or those of Hart (1957).

It is possible that some of the effects of thyroxine treatment may be influenced by such factors as plane of nutrition and the presence or absence of the various hormones involved in pregnancy and lactation. More definitive evidence is required on these factors as well as on the stimulation of wool growth at various times of the year. This latter point is of importance as Hart's (1955) theoretical interpretations of light effects on wool growth are based on a lack of action of somatotrophic hormone given in November and December. The difference in solubility between batches of thyroxine implants may also have influenced the experimental results of different workers.

There appears to be little point in discussing further the variations in the action of implanted thyroxine on wool growth until the results of more adequate experiments, suitably analysed, are available.

The last part of the present experiment was included in order to find out how little thyroxine was necessary to produce a measurable increase in heart rate which has been shown to be a sensitive indicator of thyroxine activity. This experiment, carried out on the six non-tracheotomised ewes, showed that the effects of 0.75 mg thyroxine/day could be detected. The experiments of Ferguson (1958) and Myers and Ross (1959) have also shown that heart rate is a useful indicator of hyperthyroidism.

It is of interest to note that L-thyroxine is almost insoluble in a neutral solution (Evert 1959). Although the thyroxine solution was alkaline when injected in the present experiment, it is likely that it would be rapidly neutralised in the body. This would result in the thyroxine solution changing to a suspension. Such a change could slow down the rate of uptake of the thyroxine and so explain the time lag between the first injection and the first observed treatment effects. Similarly, it could explain the continuation of the treatment effects for several days after the last 5 mg thyroxine injection was given (see Fig. 2).

Dukes (1947) has indicated that the resting heart rate of sheep is normally 70–80 beats/min, and similarly Wirth (1956) has suggested

that the normal heart rate of sheep is in the range of 70–90 beats/min. It can be seen from Table 3 that the overall mean heart rate of 80 beats/min (range 59–110) at the beginning of the experiment fell within the normal ranges. The individual ewe means varied considerably and two fell outside the larger range of Wirth. However, by the end of the experiment, the mean heart rate of the six ewes still being studied had fallen to 59 beats/min (range 43–70) which was a mean drop of 18 beats/min for the six ewes concerned.

Two factors were probably involved in the lowering of heart rate during the experiment. As the animals became more accustomed to being handled, the mean heart rate dropped and the difference between successive observations decreased. This could be explained by the observation (Dukes 1947) that excitement influences heart rate, as the ewes became very much quieter as the experiment progressed. It may also be noted that a rapid drop occurred immediately after 26 June, the last day on which the animals were fed the combined ration of oats, peas, and hay. From this date onwards the animals were fed only hay. It can be seen from Table 3, by comparing column M_3 with column M_4, that the four non-injected ewes showed reduced heart rates of 10 beats/min over a period of a few days after the dietary change. Similar effects of nutrition on heart rate have been noted in cattle by Ritzman and Benedict (1938).

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EMBRYONIC LOSS FROM LATE BREEDING SEASON MATINGS OF EWES

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Summary

Information on the high loss of fertilised ova, 40% being abnormal, which occurs in ewes mated during the latter part of the breeding season is presented.

The results have been obtained from mating sixty middle-aged ewes to rams of known potency during the period 1 June to 14 August 1958.

Seventy-seven tubal and uterine ova were recovered from eighty-five ovulations.

Introduction

The study of the breeding season of the ewe shows that there is a definite pattern of reproductive activity and fertility. The ovulation rate has been shown to rise to a maximum during the first few oestrous cycles of each breeding season and then to fall during the later cycles of the season. This has been indicated by Hammond Jr. (1944) working with Suffolk-cross × Border Leicester Cheviot ewes; Watson (1952) with Merino ewes; Hulet *et al.* (1956) working with Shropshires and Hampshire ewes; Hart, Dutt, and Laffey (unpublished data) using ovum recovery and corpora lutea counts of 1100 reproductive tracts from various pure and cross-bred ewes collected throughout the breeding season.

The fertility level has been more difficult to analyse. Hammond Jr. (1944) found a definite decline in the fertility of the ewe late in the breeding season. He was, however, unable to say whether the fault lay with the ewe or with the ram. Dutt (1951) showed an estimated loss of 20% of embryos from early-season matings whilst Hart (1956) also showed that a proportion of all ova shed were abnormal and not able to be fertilised. Hulet *et al.* (1956) also found a highly significant increase of fertility with the advance of the season from first service onwards.

The purpose of this trial was to provide more specific information on the rate of embryonic loss from late-breeding-season matings when using rams of tested potency.

Materials and Methods

Ewes. The trial was commenced on 1 June and concluded on 14 August. Samples were taken over five 15-day periods. Halfbred ewes, brought into the College as killer ewes, were run with a raddled entire ram. Nutrition was at maintenance level. Twice weekly the tupped ewes were drafted from the flock and killed. The reproductive tracts

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were removed from the ewes immediately after killing and taken straight to the laboratory where they were examined. Corpora lutea and egg counts, together with assessment of age of ovulation were made. The ova were recovered by flushing the tube or uterus with warm (37°C) physiological saline. Previous experience had shown that this procedure for egg recovery resulted in no observable damage to the ovum, this is supported by successful transfers of fertilised eggs recovered in this manner (Hart, Thomson, Dutt, and Laffey 1957; unpublished data. There appears to be no reason to suspect egg-handling techniques as a source or cause of abnormal changes occurring in ovum anatomy. The ova were then examined under low and high power of the microscope for zona pellucida and blastomere detail. Only those with spermatozoa in the zona were regarded as fertilised. The necessity to define a single-cell fertilised sheep's ovum for this and other experiments has always presented a certain amount of difficulty. However, as the result of examining many hundreds of sheep's eggs and carefully checking all those recovered from test fertile matings we have formed the conclusion that invariably where natural matings have been used, all young fertilised eggs showing cleavage will have sperms or sperm residues embedded in the zona pellucida. Not yet have we found, over a period of five years, any egg showing normal fertilisation cleavage which failed to conform to the above description. It has therefore seemed reasonable to classify a single-cell egg with sperm adhering to the surface, and embedded in the zona pellucida, as being fertilised. Additional support for this assumption, is that single-cell eggs of this nature, which were transferred by Hart and his co-workers in 1957 (unpublished) resulted in live lambs being produced.

Rams. The rams used were those kept at the College for research purposes. Each ram was used for a week and then spelled. Both the outgoing and incoming rams were semen-tested, and palpated for epididymitis. The semen was collected by the electro-ejaculator method except in those cases where samples were difficult to obtain, when an artificial vagina, inserted into the vagina of an oestrous ewe, was used and the ram allowed to serve naturally.

The semen was judged on:

(1) the motility of spermatozoa,

(2) the concentration of spermatozoa,

(3) the percentage motility of spermatozoa.

(4) the percentage of abnormal sperm.

Any ram not giving a satisfactory semen sample was discarded. A satisfactory sample was one with spermatozoa of good motility and present in a concentration greater than 350×10^4 per cubic millimetre.

Smears were taken of semen and stained with a modified Ziel Nielson stain and examined for brucellosis, all semen smears proving negative.

All rams used were palpated for epididymitis, with negative results. In all cases when many stimulations were necessary for the ram to

serve, the semen sample was unsatisfactory with low motility and many abnormal spermatozoa.

Results and Discussion

Ewes. The assessment of age of the corpora lutea yielded some interesting results. From experience with mid-season ewes it has been possible to determine the age of any early ovulation to within twenty-four hours by the internal and external examination of the corpus luteum. Estimate of age is based on the amount of luteal tissue in the follicular space. With these late-breeding-season ovulations the same criteria gave very different results. Normally, in mid-season breeding the corpus luteum of a four-day-old ovulation has commenced to decrease in vasculation, the cavity has almost disappeared, and the amount of luteal tissue formed is considerable. The ova at this stage are in the uterine horn and, if fertilised, are in the sixteen-thirty-two or morula stage of development. However, during the late-breeding-season period four-day ova of comparable midseason cell development were often found linked with corpora lutea showing large cavities, very small amount of luteal tissue, and heavy vasculation. From these results it would appear that either (1) the rate of development and speed of the ova down the tube is greater late in the breeding season or (2) the amount and rate of formation of luteal tissue formed decreases late in the season. As far as could be judged from this field trial the rate of travel of the ova seemed to be normal. However, the matings were not controlled so that this has yet to be tested.

The basis for determination of a fertilised ovum was, as mentioned previously, the presence of sperms or sperm residues embedded in the

TABLE 1. DATA FOR THE FIVE 15-DAY PERIODS

Period	Date 1958	No. of Ewes with Fertilised Ova	Ovulations	Recovered Ova	Abnormal Ova	Types of Abnormalities	
1	1 June	27	42	38	14	Broken zona	5
						Irregular development	8
						Unfertilised ova	1
2	16 June	7	12	8	2	Broken zona	1
						Irregular development	0
						Unfertilised ova	1
3	1 July	15	19	19	10	Broken zona	4
						Irregular development	3
						Unfertilised ova	3
4	16 July	2	3	3	0	——	
5	31 July	9	9	9	5	Broken zona	3
						Irregular development	1
						Unfertilised ova	1
	Total	60	85	77	31 (40%)	Broken zona	13 (41.9%)
						Irregular development	12 (38.7%)
						Unfertilised ova	6 (19.4%)

zona pellucida, and the results from these ova only are presented. Fertilisation failure has been assumed only in those ova from multiple ovulations in which the twin was fertilised. This was necessary because all matings took place under field conditions, and it was not possible to use any other criteria for postulating that an egg had been actually exposed to contact with fertile sperms.

Fig. 1.—Abnormal 3-day tubal ovum with broken zona pellucida. Sperm embedded in zona indicate ovum had been fertilised.

The results for the five 15-day periods are to be seen in Table 1. The small numbers in Period 4 are due to flocks having been in advanced stages of pregnancy when the individuals were killed.

The percentage of abnormal ova for the entire five periods was 40. These were broadly classified as follows:

(1) Broken zona pellucida tubal ova with sperm in the zona pellucida. These represented 41.9% of the abnormal ova (Fig. 1).

(2) Tubal ova in which cell development was irregular. The blastomeres were usually at a degenerative state with some breakdown

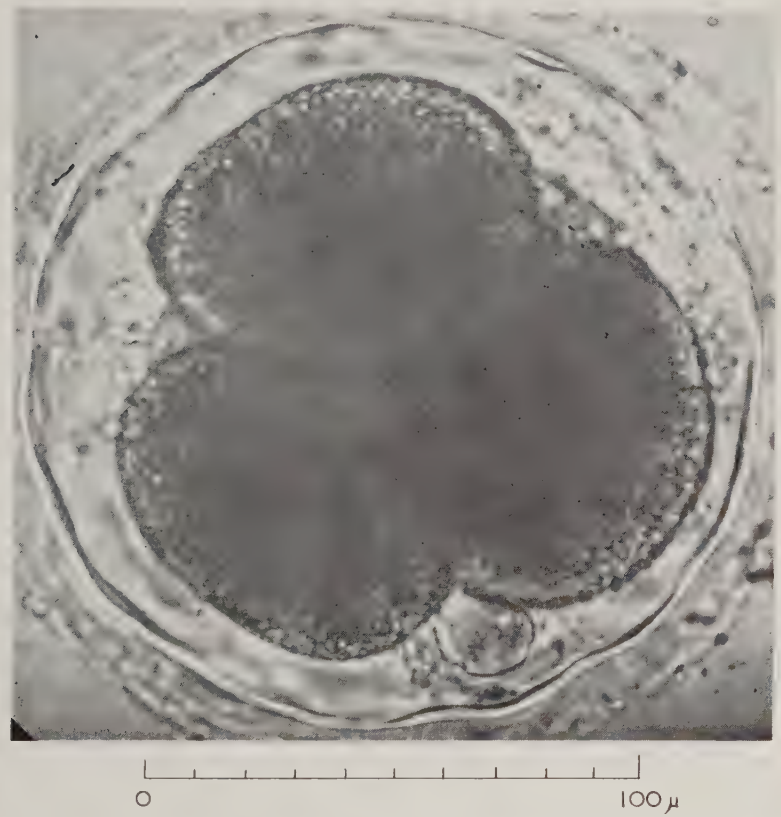

Fig. 2.—Abnormal 2-day fertilised tubal ovum showing vacuolation, and blastomere membrane break-down with exudation into perivitelline space.

of the membrane, and general exudation of material into the perivitelline space (Fig. 2).

(3) Uterine ova in which cell development was irregular (Figs. 3, 4).

(4) Those ova in which spermatozoa had failed to penetrate the zona pellucida.

On present data it is difficult to advance any theory as to why this high incidence of abnormal ova should occur in the latter part of the breeding season. It certainly must be accepted that there is an increase, as Hart's (1956) figure for the main part of the breeding season was 17.3% abnormal. It should be noted that in Hart's early classification the incidence of broken zona pellucida among the abnormal group was apparently not high enough to be mentioned. In the present investigation by far the greatest proportion were classified in this category.

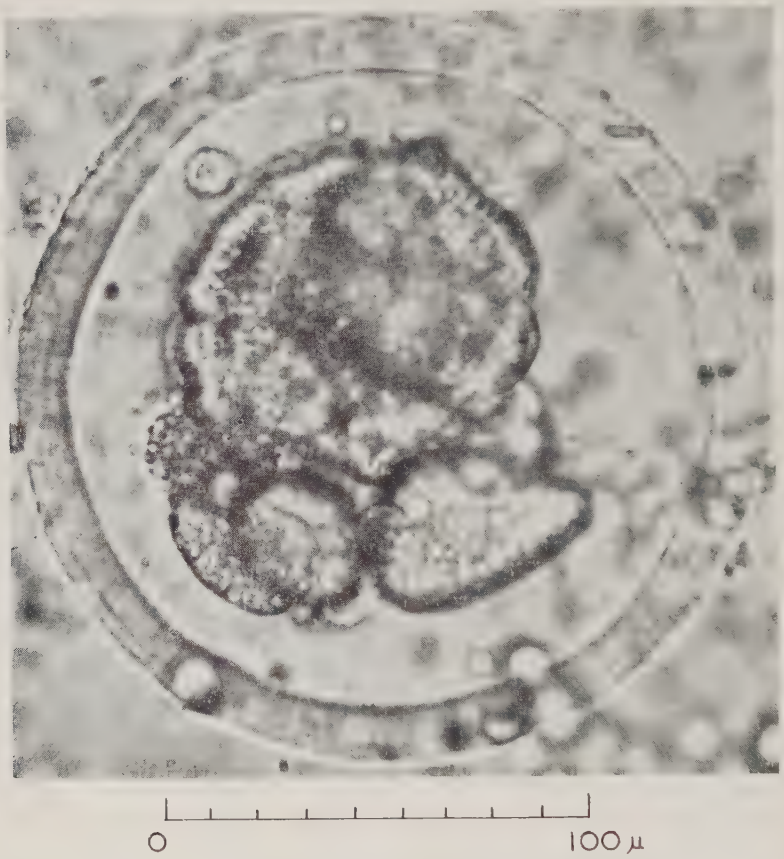

Fig. 3.—Abnormal 5-day fertilised uterine ovum showing irregular blastomere development.

The evidence of the corpora lutea and ova development and travel being out of phase compared with their relationship during the earlier part of the breeding season is also noteworthy. It is possible that this influence on the intensity of the progesterone phase of the ovulatory cycle may result in an unsuitable tubal-uterine environment in which the zona of the egg reacts by cracking. The other possibility is that the egg travels too fast and so finds itself in the uterus before it has had time to complete an "egg capacitation" process analogous perhaps to sperm capacitation. However, as previously pointed out, the data at present available do not allow for a full and detailed explanation of the situation. The main purpose of this paper is to indicate that during the latter portion of the ewe breeding season an extremely high proportion of eggs shed are not fertilised and are abnormal. Also, that the percentage of abnormal eggs is at least twice as high as it is in the earlier stages of the breeding season.

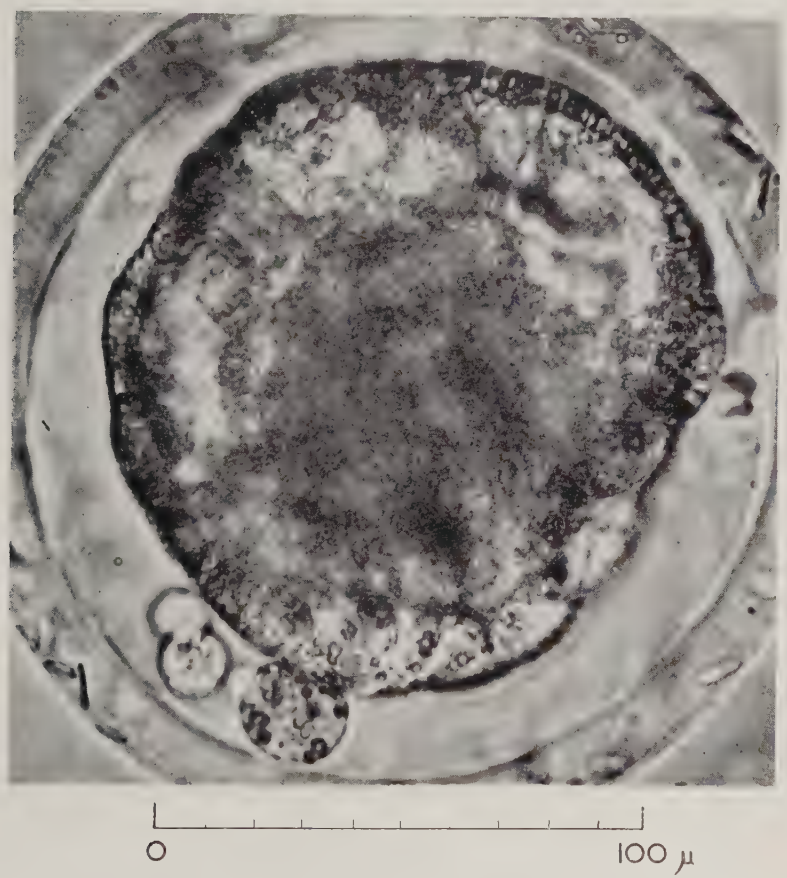

Fig. 4.—Abnormal 7-day fertilised uterine ovum showing irregular development with some cells completely detached.

To some extent these points are of course purely academic, as under normal breeding conditions it is unlikely that many ewes would be mated during this period. However the gradual rise in abnormal egg production during the breeding season to its peak of 40%, as shown by this investigation, indicates at least one possible explanation for the lower lambing figures invariably obtained from ewes mated during the latter stages of the breeding season.

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CHEMICAL INVESTIGATIONS ON BITTER PIT OF APPLES

I. PHYSICAL AND CHEMICAL CHANGES IN LEAVES AND FRUITS OF COX'S ORANGE VARIETY DURING THE SEASON

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Summary

Fruits of Cox's Orange variety have been examined for size, and dry matter content, and have been analysed for minerals, nitrogen and sugars contents from just after petal fall to beyond commercial maturity, for two growing seasons. Leaves from fruiting spurs and from leaders were also analysed.

Appreciable differences, especially in minerals and nitrogen contents, were found for the two seasons. Nitrogen, calcium, magnesium, potassium, sodium and phosphorus percentages decreased on the fresh and dry-matter basis, but accumulation of these elements occurred steadily, with growth of the fruits. On the other hand leaves increased in ash, calcium, and magnesium contents, but decreased in potassium, sodium, phosphorus and nitrogen, with increasing age. Expressed on the fresh basis, leaves were much richer than fruits in all these elements, especially in the latter half of the season.

On both fresh and dry basis, fructose and sucrose increased but glucose remained relatively constant, with increasing size, although the amounts of these sugars on the per-fruit basis rose rapidly. Leaves were relatively poor in sugars. In the leaves, sucrose was relatively high when fructose was low, and vice versa.

The data recorded are regarded as fundamental for the study of chemical composition in relation to bitter pit.

Introduction

Under various local names, of which bitter pit is probably now the one most widely used, a world-wide defect of apple fruits affecting a number of varieties in varying degrees of severity has been known for a number of years. Up to the present no definite cause for or means of control of it has been known. The defect is made manifest by the appearance of brown cork-like areas in the flesh of the fruit, usually just under the skin, causing visible depressions. These brown areas may however extend throughout the flesh; they are mostly individual and do not coalesce. At times these "pits" may occur in fruits on the tree, but the disease is essentially a storage trouble, whether of fruit held in cool store or under atmospheric conditions. Such affected fruit may be commercially worthless, by reason not only of appearance but also of a bitter flavour of the fruit.

Many causes have been suggested by a great number of investigators into the bitter-pit problem. It appeared that this defect of the fruit was due to physiological causes, of which withdrawal of water was

a favourite suggestion for the collapse of cell structure that occurs in the affected tissues. In practically all cases starch granules are found in such tissues. Some antecedent cause is therefore suggested which prevents normal hydrolysis of starch in the cells. It is remarkable too that this collapse of normal function mostly occurs at the ends of vascular bundles.

In the past there has been a certain amount of sporadic, but not very detailed, chemical work done on this fruit problem. McAlpine (1911-12) in his classical work on bitter pit quotes a few analyses which show that pitted fruits have higher dry matter, ash, and reducing sugars contents than healthy fruits; on the other hand, acid and sucrose contents were relatively low. A positive conclusion in respect of mineral contents was reached by De Long (1936), who found that especially the skin, but also the flesh, of affected fruit was much lower than in healthy fruit. He says, ". . . a low calcium content in apples affected with this disorder may arise as a result of competition between leafy shoot and fruit tissues for this element during these early stages of growth. Possibly this apparent calcium deficit may play a part in the origin of the lesions typical of this disorder." Unfortunately it appears that this work was never followed up. Garman and Mathis (1956) concluded "that unbalanced mineral ratios have a profound effect on Stippin or Baldwin Spot in Connecticut. It would appear that the critical element is calcium and the unbalanced condition lies between calcium and magnesium; or calcium and magnesium plus potassium. There is also a definite connection between the amount of spot and the leaf/fruit calcium ratio." They do not refer to the work of De Long. They were able to obtain some control of their ailment, which is the same as bitter pit, by using calcium nitrate, which in the present authors' view complicates the question by introducing a nitrogen effect, applications of the latter element being known to increase incidence of the ailment. Garman and Mathis apparently lean also to the idea that boron is involved in this matter.

In view of the poverty of detailed chemical data on the bitter-pit problem it was decided to institute a programme of chemical work on bitter pit as it affected varieties of apple grown in Nelson. At harvest in the first few months of 1957, samples of susceptible and not so susceptible varieties were obtained. In the next year further samples of several varieties were taken for analysis. Also field experiments were established in the 1957-58 season from which samples of developing Cox's Orange fruits and leaves were obtained periodically throughout the growing period. In addition a trial using different mineral salts as sprays on Cox's Orange was started in the 1957-58 season and continued in the 1958-59 season. Cox's Orange was chosen because it is very susceptible to bitter pit and is also a valuable variety commercially.

The expectation therefore is that eventually it will prove possible to correlate susceptibility to and severity of incidence of bitter pit with the chemical composition of fruit and leaves. It may also prove possible to use chemical data obtained on fruits and leaves for predicting

incidence of pitting in a given season. Because bitter pit is the result of a physiological disturbance such a chemical approach to the problem appears to be justified.

In the present paper an account is given of inquiry into the physical and chemical changes occurring in fruits and leaves of Cox's Orange variety during the two seasons, 1957-58 and 1958-59.

EXPERIMENTAL

Weather Conditions

There were no facilities at the experimental orchard for taking meteorological records, but there is an official station of the Meteorological Service at Nelson Airport about a mile west of the orchard. Data from this Station have been used.

Mean monthly maximum and minimum temperatures, variation from the average mean monthly temperature, rainfall and its variation from the average monthly figure, highest daily rainfall, and the day on which it fell, in each month, and the hours of bright sunshine for the month, for the periods Aug. 1957 to March 1958 and for Aug. 1958 to March 1959 are all shown in Table 1.

In the former season there was little variation in mean temperature over the spring and early summer periods, but the late summer and early autumn months averaged nearly three degrees warmer than the average of a number of years. Rainfall was deficient in early spring of this season, higher than normal over the main spring period, dry in early summer, wet in February and dry again in March. There were no excessively large falls of rain in any one 24-hour period until the reading of 2.19 in. of 17 February 1958. Bright sunshine was relatively high except in the dull month of February 1958 with only 128 hours of sunshine. Thus over most of the season rainfall, temperature, and sunshine were fairly normal but the harvest month of February was abnormally warm, wet, and dull.

The season of August 1958 to March 1959 is quite different from the previous one, being unusually warm especially over the growing and harvest periods, and generally deficient in rainfall over those periods. Until the end of November the deficiency in rainfall amounted to 5.42 inches; this covers the period when blossoming, setting of fruit and early developments of fruit and leader growth occur. December 1958 was a wet month, the total of 5.78 inches being 2.94 inches above average. January and February 1959, when rapid development of fruit and extension growth of leaders take place, were unusually warm and dry. The highest temperature of the season, 84.1°F, was recorded on 27 January. In each month from November 1958 to March 1959 the maximum temperature was over, or nearly, 80°F. Hours of sunshine was relatively low in October and December 1958, but was high in November 1958 and in January and February 1959. This season was much sunnier than the first one, but like the latter it was warmer and drier than normal.

TABLE 1. Monthly Weather Records for Aug. 1957 to Mar. 1958 and Aug. 1958 to Mar. 1959

Month	Monthly Mean		Mean Temp diff. from av. (°F)	Rainfall (in.)	Rainfall diff. from av. (in.)	Maximum Fall		Bright Sunshine (hours)
	Max. °F	Min. °F				Inches	Day	
1957 –								
Aug.	56.2	38.9	+2.8	2.07	—1.07	0.94	11	164
Sept.	58.7	39.9	+1.1	1.57	—1.59	0.34	27	200
Oct.	60.5	43.7	—0.1	3.62	+0.28	0.84	12	224
Nov.	63.9	48.9	+0.9	4.16	+1.43	0.78	9	196
Dec.	66.4	50.4	—1.0	2.15	—0.69	0.61	17	214
1958—								
Jan.	69.4	53.5	—0.2	1.30	—1.54	0.62	4	260
Feb.	70.8	58.2	+3.3	5.82	+3.20	2.19	17	128
Mar.	68.8	53.2	+2.6	1.20	—1.49	0.32	15	191
1958—								
Aug.	55.3	36.7	+1.2	2.57	—0.57	0.55	28	177
Sept.	57.1	37.7	—0.8	2.10	—1.06	1.89	13	252
Oct.	62.0	46.8	+2.2	1.84	—1.50	0.41	28	158
Nov.	66.4	47.5	+1.5	0.44	—2.29	0.22	15	276
Dec.	68.9	56.3	+3.2	5.78	+2.94	1.38	23	199
1959—								
Jan.	72.3	56.2	+2.6	1.19	—1.65	0.59	29	295
Feb.	72.2	54.3	+2.0	0.41	—2.21	0.34	9	239
Mar.	69.2	53.3	+2.8	5.51	+2.82	2.04	17	202

Note: Data extracted from N.Z. Meteorological Service monthly reports in N.Z. Gazette.

Soil Analysis

Samples of soil down to a depth of 18 in. have been obtained from 4–5 ft out from the trunks of the trees, this being the area over which most of the annual applications of fertilisers have been applied, and from the central points where the inter-row areas cross. The effect of the fertiliser applications is seen in the lower pH values and percentage base saturation, and in the increased citric-acid-soluble potash and phosphate of the soil nearer the trees. Lack of penetration of phosphate below the cultivable layer is clearly shown. Loss on ignition, total nitrogen, and organic carbon decrease with increased depth of sampling; for corresponding depths, the two sets of samples show very similar figures for these determinations. The C/N ratio is about 15 in the top soil and decreases in value, more rapidly close to the trees, with depth of sampling. Cation-exchange capacity, exchangeable calcium,

magnesium, and potassium, and base saturation, all decrease with increasing depth of the soil. All the figures are very satisfactory. Here again the effect of concentrating the fertiliser about the trees is shown. Greater depth of cultivation was possible between the rows and this increased the penetration of phosphorus and calcium into the 6–12 in. layer with a consequent rise in the base-saturation figure. It is interesting to note the close correlation between the two methods, with citric acid extraction and as exchangeable, used to measure available potassium; the former on the average gives values 78% of the latter. The two figures approach one another even more closely in the top layer.

This soil, according to the analytical data obtained, is well supplied with nutrients in the upper layers but in the regions where the more deeply penetrating roots would be it is very low in available phosphorus and low in available potassium.

Samples of developing fruits and of leaves, from fruit-bearing spurs in both 1957-58 and 1958-59 seasons and from basal portions of leaders in 1958-59, were obtained throughout the season from a unit of 12 Cox's Orange apple spur-pruned trees at the Annesbrook Orchard of the Cawthron Institute, commencing about a fortnight after petal fall and continuing to maturity. For the earliest samples 100 fruits were taken, the number being progressively reduced as the fruit grew, but at no time were less than 25 fruits used per sample. Fresh weights and percentages of dry matter of the fruits were determined, the dried material being retained for chemical analysis. On the leaf samples percentages of dry matter were determined but no records were kept of weights of leaves.

The following chemical determinations have been carried out on the fruit and leaf samples: total ash, ignition at 450–500°c; calcium, as oxalate using permanganate titration; magnesium, as hydroxyquinolate with titration finish; potassium and sodium, by flame photometer; phosphorus, colorimetric vanadate; total nitrogen, sulphuric-salicylic acid digestion; water-soluble reducing sugars, by Somogyi method; glucose, by hypo-iodite; fructose, as difference between total reducing sugars and glucose; sucrose, by dilute acid hydrolysis and determination of increase in reducing sugars. All the data have been expressed on the dry-matter basis, and as the element for the minerals and nitrogen.

Calculations have been made from the data for the amounts of the several constituents in the fresh material as sampled, and for determination of the uptake of minerals and nitrogen and the accretion of dry matter and sugars per fruit during growth.

Results

Physical Changes

A record of the changes in weight and percentages of dry matter of the fruits is given for the two experimental periods in Table 3, and corresponding data for leaves in Table 4. It will be noticed that in both seasons samples were taken at about 10-day intervals early in the season and at somewhat longer ones later.

TABLE 2. Analyses of Soil Samples from the Experimental Areas

Position	Depth of Sampling inches	pH	Loss on Ignition %	Total N %	Organic C %	Citric-acid-soluble %		Exchangeable Cations me. %				Base Saturation %
						P_2O_5	K_2O	C.E.C.	Ca	Mg	K	
4–5 ft from trunks of tree	0– 6	6.24	8.1	0.184	2.78	0.063	0.042	16.51	10.50	1.28	1.08	76.5
	6–12	5.18	6.6	0.101	1.28	0.004	0.021	14.79	6.79	1.14	0.69	55
	12–18	4.75	7.4	0.083	0.86	0.001	0.010	19.48	7.21	1.87	0.34	43
Centre of rows	0– 6	6.72	8.0	0.186	2.84	0.067	0.032	16.11	12.98	1.11	0.83	91
	6–12	6.01	6.9	0.109	1.52	0.007	0.008	13.29	8.47	0.90	0.22	71
	12–18	5.13	6.7	0.072	0.84	0.001	0.004	14.47	5.50	1.35	0.08	45.5

In the 1957-58 season ten samples were taken over 110 days, and in 1958-59 thirteen samples over 144 days; it may be noted that the fruit was too mature for cool storage, and was fully tree-ripe, when sampling ceased in 1959. During November, with a doubling of the accretion of dry matter per fruit in 10 days, in December and January with a doubling in about 20 days, rapid growth of the fruits took place; these are only broad groupings, however, applicable to both seasons. From the beginning of December to the normal picking date of about 19 February the apples increased their dry-matter contents ten-fold.

Percentage of dry matter in the fresh material increased, except for the relatively high figure of 13.2% for the fruitlets on 14 November 1957, progressively from 8.2% to 13.7% in the first season, and from 10.9% to 15.4% in the second (omitting the apparently aberrant 18.4% of 4 February). It may be noted that the dry-matter contents, especially early in the season, are higher in the second year; the very dry weather of that season may explain this. Rate of accretion in dry matter has been relatively greater therefore than the rate of increase in weight. Fruit of the second season was about double the weight of that of the first at time of commercial picking about mid February.

TABLE 3. WEIGHT AND DRY-MATTER CHANGES IN COX'S ORANGE FRUITS DURING GROWTH

Date of Sampling	Days from Start of Sampling	Fresh Weight g	Dry Weight g	Dry Matter per cent
1957-58 Season				
14.11.57	0	3.86	0.51	13.2
26.11.57	12	5.55	0.45	8.2
5.12.57	21	7.75	0.70	9.1
13.12.57	29	12.38	1.16	9.4
24.12.57	40	17.96	1.64	9.1
7. 1.58	54	—	3.23	—
22. 1.58	69	52.5	7.17	13.7
8. 2.58	86	76.6	9.68	12.6
19. 2.58	97	76.2	8.98	11.8
4. 3.58	110	79.2	10.86	13.7
1958-59 Season				
10.11.58	0	4.43	0.52	11.7
20.11.58	10	9.99	1.09	10.9
28.11.58	18	16.75	1.86	11.1
8.12.58	29	30.22	3.54	11.7
17.12.58	40	44.12	3.88	8.8*
29.12.58	50	57.9	6.43	11.1
7. 1.59	59	76.8	9.65	13.2
19. 1.59	71	95.2 ?	14.09	14.8
4. 2.59	87	125.6	23.11	18.4*
18. 2.59	101	142.6 ?	19.62	13.8
4. 3.59	115	144.0 ?	19.58	13.6
18. 3.59	129	161.4	23.89	14.8
2. 4.59	144	154.8	23.84	15.4

* Aberrant figure?

It is known that larger fruit is generally more susceptible than smaller to bitter pit; but the relative susceptibility will be shown in later papers to be related also to chemical composition. In the present paper the chemical data for the leaves and fruit will be considered purely as a physiological study.

Because of the close liaison that must exist between leaves and fruit while both are growing rapidly during the season, and because of the known build-up of nutrient material in both leaves and fruit as the season progresses, knowledge of changes in the leaves may be important in any approach to the bitter-pit problem. Table 4 gives the percentages of dry matter in fruit-bearing spur leaves of the 1957-58 season, and in spur leaves and basal leaves of leaders in the 1958-59 season. For the first season the percentage of dry matter increased from an early minimum of 26.1 to a mid-season maximum of 50.3. At the end of the season the percentage had fallen to 31.5. In the second season the spur leaves increased from 32.4% on 10 November 1958, through an aberrant (?) figure of 30.4% on 8 December, to a maximum of 42.5% at about picking-time in February 1959; a fall to 38.3% then occurred. Comparing the data for the two seasons it is clear that, except at the beginning and end of the season, leaves of the 1957-58 season had much higher dry-matter contents than those of the 1958-59 season, in spite of the dryness of the latter. Some damage to the leaves by sprays in the first season may be related to this fact. With the basal leaves from the leaders a relatively steady increase in dry matter content occurred throughout the season, the first sample

TABLE 4. Changes in Percentages of Dry Matter of Cox's Orange Leaves During the Season

1957-58		Spur Leaves	1958-59		Spur Leaves	Leader Leaves
Date of Sampling	Days from Start of Sampling	Dry Matter %	Date of Sampling	Days from Start of Sampling	Dry Matter %	Dry Matter %
14.11.57	0	28.9	10.11.58	0	32.4	26.7
-	—	—	20.11.58	10	33.4	29.2
26.11.57	12	26.1	28.11.58	18	35.0	42.0
5.12.57	21	47.3	8.12.58	29	30.4	32.3
13.12.57	29	43.4	19.12.58	40	33.1	34.6
24.12.57	40	49.3	29.12.58	50	34.3	36.1
7. 1.58	54	50.3	7. 1.58	59	35.6	36.9
22. 1.58	69	47.1	19. 1.59	71	36.9	40.5
8. 2.58	86	42.0	4. 2.59	87	33.0	35.0
19. 2.58	97	35.7	18. 2.59	101	42.5	43.2
4. 3.58	110	31.5	4. 3.59	115	38.3	40.5

having 26.7% and a late season sample 43.2%. This maximum figure was found on the same date as the maximum for the spur leaves. Except for the first two samples in November, the leader leaves were higher in dry matter than the corresponding spur leaves, but these leader leaves of 1958-59 were not as high in dry matter as most of the spur leaves of the earlier season. Both spur and, especially, leader leaves have increased their dry-matter content in parallel with the changes in the dry-matter contents of the fruits of the 1958-59 season.

TABLE 5. MINERAL AND NITROGEN CONTENTS OF DEVELOPING COX'S ORANGE APPLES
(% of D.M. for the seasons 1957-58 and 1958-59)

Date of Sampling	Days from Start of Sampling	Total Ash	Ca	Mg	P	K	Na	N
14.11.57	0	6.60	0.28	0.20	0.31	2.27	0.017	2.89
26.11.57	12	6.29	0.25	0.20	0.24	1.92	0.017	2.46
5.12.57	21	5.03	0.21	0.15	0.22	1.72	0.016	2.16
13.12.57	29	4.57	0.17	0.13	0.17	1.65	0.019	1.90
24.12.57	40	4.46	0.15	0.11	0.16	1.55	0.022	1.62
7. 1.58	54	3.48	0.10	0.08	0.11	1.10	0.026	1.19
22. 1.58	69	2.70	0.053	0.056	0.080	1.13	0.024	0.77
8. 2.58	86	2.54	0.042	0.056	0.085	0.98	0.018	0.76
19. 2.58	97	2.35	0.032	0.050	0.069	0.81	0.013	0.74
4. 3.58	110	2.27	0.023	0.042	0.059	0.80	0.021	0.61
10.11.58	0	6.43	0.31	0.16	0.23	1.90	0.017	2.17
20.11.58	10	4.77	0.21	0.13	0.19	1.74	0.015	1.79
28.11.58	18	3.70	0.14	0.097	0.14	1.34	0.010	1.38
8.12.58	29	3.10	0.088	0.072	0.097	1.00	0.012	0.99
17.12.58	40	2.58	0.070	0.058	0.080	0.87	0.011	0.88
29.12.58	50	2.37	0.060	0.050	0.066	0.72	0.011	0.92
7. 1.59	59	1.95	0.039	0.039	0.044	0.83	0.010	0.64
19. 1.59	71	1.73	0.030	0.031	0.029	0.70	0.011	0.53
4. 2.59	87	1.68	0.021	0.033	0.039	0.53	0.008	0.41
18. 2.59	101	1.96	0.029	0.037	0.038	0.67	0.013	0.43
4. 3.59	115	2.02	0.027	0.037	0.052	0.75	0.015	0.43
18. 3.59	129	1.88	0.020	0.033	0.046	0.65	0.014	0.52
2. 4.59	144	1.84	0.021	0.033	0.052	0.70	0.016	0.43

Chemical Changes

Fruit. Results of determinations of mineral and nitrogen contents of the developing fruits during the two experimental seasons are given in Table 5.

It is seen from these data that all the constituents determined, except sodium, started off at relatively high values, which fell steadily at a rate of about 10% of their absolute values in 1957-58 and at about 25% in 1958-59, for each 10-day period until the end of January in both seasons. Sodium content, on the other hand, rose to a maximum value early in January 1958 but in the second season such a maximum is not to be seen.

There are appreciable differences in the levels of the several constituents at all corresponding dates in the two seasons. In 1957-58 all the constituents, except for one instance with sodium, given in Table 5 are higher than in 1958-59, but in both seasons the levels of all constituents fall to low values. Thus on 18-19 February the percentages were for the first and second seasons respectively: total ash, 2.35 and 1.96; calcium, 0.032 and 0.029; magnesium, 0.050 and 0.037; phosphorus, 0.069 and 0.038; potassium, 0.81 and 0.67; sodium, 0.013 and 0.013; nitrogen, 0.74 and 0.43.

A further very interesting point concerns the percentages of calcium and magnesium in the dry matter. In both seasons calcium was present in larger amount than magnesium early in the season, this difference being especially noticeable in the second season. It will also be seen that the calcium content falls more rapidly than the magnesium one, consequently there comes a time when these two elements are present in equal amount (as percentages); this occurred about 22 January 1958 and on 7 January 1959. After these dates there was more magnesium than calcium in the dry matter.

Attention may be drawn to an apparent build-up of nutrients in the fruit of the 1958-59 season as they approach a condition of tree-ripeness. This build-up is especially marked with total ash, phosphorus, potassium, and sodium.

In addition to the mineral and nitrogen data, there are figures available for the glucose, fructose, and sucrose contents of the fruits, these being expressed as percentages of the dry matter in Table 6.

During the drying of some of the samples of the 1957-58 season discolouration occurred, possibly due to overheating, so that these, covering most of December 1957, have not been reported in Table 4. The data available for the first season show that as the apple grows the percentages of fructose and sucrose increase markedly but glucose remains relatively steady. These conclusions are confirmed by the data for the second season, as shown in the lower section of Table 6. During rapid growth of the fruit (November and December) the sucrose content remains low but fructose increases rapidly. In the final stages of growth, in both seasons, sucrose and fructose were present in approximately equal amounts in the dry matter. The rise in glucose and fall in fructose and sucrose with full maturity at 2 April 1959 is interesting, but is not discussed further.

TABLE 6. SUGARS CONTENT OF DEVELOPING COX'S ORANGE FRUITS IN THE 1958-59 SEASONS

(% of D.M.)

Date of Sampling	Days from Start of Sampling	Glucose	Fructose	Sucrose
14.11.57	0	4.8	3.7	2.5
26.11.57	12	5.8	7.2	0.5
5.12.57	21	7.4	8.7	N.F.
8. 2.58	86	4.4	17.8	4.6
19. 2.58	97	5.5	18.9	11.4
4. 3.58	110	8.3	24.4	23.4
10.11.58	0	4.2	6.4	1.0
20.11.58	10	6.6	8.7	3.1
28.11.58	18	7.1	10.7	2.1
8.12.58	29	8.6	16.0	1.0
19.12.58	40	9.6	23.2	3.5
29.12.58	50	7.4	19.9	3.7
7. 1.59	59	5.6	22.4	9.0
19. 1.59	71	4.7	20.0	10.2
4. 2.59	87	5.7	23.6	13.2
18. 2.59	101	6.3	24.0	14.1
4. 3.59	115	6.5	25.9	21.7
18. 3.59	129	8.4	26.2	25.7
2. 4.59	144	9.6	17.2	20.9

N.F. means "not found."

Leaves. Data for the mineral and nitrogen contents of leaves sampled on the same dates as the fruit are given in Table 7, the figures therein being expressed as percentages of the elements, excepting the ash, in the dry matter. It will be remembered that in 1957-58 spur leaves were obtained, but that in 1958-59 both spur and leader leaves were sampled.

The percentages of ash, calcium, and magnesium increase, but phosphorus, potassium and nitrogen decrease, with age of the leaves. In the spur leaves calcium content almost doubles over the season; magnesium content increases proportionately much less. Nitrogen content during the later part of November and during the whole of December remains relatively constant in both types of leaves, but decreases occur in January and February. Sodium content passed through a maximum about mid December 1957 but similar maxima were not shown in the next season. It is noticeable too that the sodium content of spur leaves was much higher in the first than in the second season. Taking the spur leaves, ash, calcium, magnesium, phosphorus, and nitrogen were higher in 1957-58 than in 1958-59 at corresponding dates, although at the end of the sampling periods, except for sodium, there were no great differences in composition between the two seasons' samples.

TABLE 7. Percentages of Ash, Mineral Elements, and Nitrogen in D.M. of Spur and Leader Leaves of Cox's Orange in Two Seasons

Season	Type of Leaf	Date of Sampling	Total Ash	Ca	Mg	P	K	Na	N
1957-58	Spur	14.11.57	6.97	1.16	0.25	0.24	1.72	0.033	3.32
		26.11.57	7.12	1.25	0.25	0.22	1.58	0.069	3.06
		5.12.57	6.55	1.28	0.23	0.18	1.25	0.073	2.63
		13.12.57	7.68	1.46	0.28	0.19	1.52	0.082	2.52
		24.12.57	8.20	1.58	0.25	0.20	1.50	0.065	2.60
		7. 1.58	8.98	1.76	0.30	0.21	1.60	0.075	2.62
		22. 1.58	8.18	1.70	0.28	0.21	1.22	0.046	2.37
		8. 2.58	8.60	1.85	0.33	0.19	1.34	0.033	2.21
		19. 2.58	9.26	1.91	0.32	0.17	1.37	0.034	2.21
		4. 3.58	9.46	2.08	0.28	0.16	1.29	0.051	2.52
1958-59	Spur	10.11.58	7.27	0.98	0.21	0.20	2.59	0.030	3.18
		20.11.58	7.34	1.03	0.21	0.18	2.26	0.031	2.61
		28.11.58	7.70	1.45	0.26	0.18	2.11	0.028	2.76
		8.12.58	7.58	1.38	0.29	0.17	2.17	0.026	2.60
		19.12.58	8.63	1.63	0.35	0.16	2.12	0.030	2.73
		29.12.58	7.69	1.48	0.32	0.14	1.94	0.023	2.42
		7. 1.59	7.99	1.63	0.31	0.15	1.88	0.027	2.45
		19. 1.59	8.36	1.68	0.29	0.14	2.09	0.024	2.45
		4. 2.59*	8.60	1.72	0.34	0.15	1.52	0.022	2.22
		18. 2.59	8.68	1.89	0.31	0.15	1.40	0.019	2.08
		4. 3.59	9.05	1.78	0.32	0.12	1.21	0.022	1.86
	Leader	10.11.58	7.32	1.21	0.23	0.20	2.24	0.025	3.12
		20.11.58	7.03	1.17	0.22	0.18	2.32	0.025	2.75
		28.11.58	7.26	1.22	0.22	0.18	2.14	0.023	2.68
		8.12.58	7.59	1.36	0.24	0.16	2.04	0.021	2.64
		19.12.58	7.66	1.39	0.26	0.16	2.07	0.023	2.65
		29.12.58	7.55	1.36	0.26	0.15	1.90	0.019	2.64
		7. 1.59	7.46	1.45	0.22	0.15	1.97	0.022	2.60
		19. 1.59	8.31	1.76	0.31	0.15	1.87	0.025	2.53
		4. 2.59*	8.19	1.66	0.28	0.17	1.41	0.017	2.49
		18. 2.59	8.16	1.61	0.27	0.15	1.35	0.018	2.17
		4. 3.59	8.01	1.52	0.26	0.13	1.20	0.020	2.07

* Leaves washed in dilute HCl to remove spray residues.

When a comparison is made between the spur and leader leaves, it is seen that there is a tendency for ash, calcium, magnesium, phosphorus, potassium, and sodium, but not nitrogen, contents, especially later in the season, to be greater in the spur leaves; in general, however, any differences are not large. The higher nutrient status of the spur leaves could have an important effect on the nutrition of the fruits growing from those spurs. It may be noted that the experimental trees were pruned on the spur system of fruiting.

As with the fruit, sugar determinations were made on the dried leaf samples; results are given in Table 8, being expressed as percentage of the dry matter.

In the spur leaves the level of glucose at corresponding dates has been similar in the two seasons, with the higher figures in the earlier

TABLE 8. SUGARS CONTENTS (% OF D.M.) IN SPUR AND LEADER LEAVES OF COX'S ORANGE IN TWO SEASONS

Season	Type of Leaf	Date of Sampling	Glucose	Fructose	Sucrose
1957-58	Spur	14.11.57	5.2	1.1	N.F.
		26.11.57	4.8	0.4	0.1
		5.12.57	5.5	1.1	0.1
		13.12.57	5.4	0.6	0.3
		24.12.57	5.1	0.3	0.7
		7. 1.58	3.9	0.0	0.6
		22. 1.58	4.0	0.4	0.4
		8. 2.58	4.0	0.3	0.2
		19. 2.58	3.1	0.2	0.1
		4. 3.58	1.7	0.1	0.0
1958-59	Spur	10.11.58	5.6	0.0	1.1
		20.11.58	5.9	0.4	1.3
		28.11.58	4.5	0.2	0.8
		8.12.58	5.7	0.3	0.8
		19.12.58	3.7	1.1	0.4
		29.12.58	4.7	1.1	0.3
		7. 1.59	4.8	0.5	0.6
		19. 1.59	4.1	0.7	0.6
		4. 2.59*	1.6	0.5	1.2
		18. 2.59	3.5	0.7	0.2
		4. 3.59	3.5	0.9	0.7
	Leader	10.11.58	5.3	0.0	1.1
		20.11.58	5.8	0.3	0.8
		28.11.58	5.0	0.1	1.0
		8.12.58	5.6	0.1	1.0
		19.12.58	3.4	1.3	0.2
		29.12.58	4.5	1.0	0.4
		7. 1.59	4.8	0.3	0.8
		19. 1.59	4.2	0.4	0.6
		4. 2.59*	1.7	0.2	0.3
		18. 2.59	3.3	0.9	0.3
		4. 3.59	4.1	0.9	0.7

N.F. means "not found."

* Samples washed in dilute HCl to remove spray residues.

part of the season. Fructose and sucrose have, however, behaved somewhat differently. Fructose was relatively high early in the first season, but not till later in the second season. Sucrose was highest in 1957-58 in late December and early January, but in the next season it was low over that period. It is noticeable that in both seasons fructose and sucrose moved inversely to one another, low sucrose being accompanied by high fructose, except at the end of the season.

Contents at corresponding dates were not greatly different for the two types of leaves of the 1958-59 season for all three sugars. Moreover it is quite clear that the leaves do not build up high amounts of sugars.

The two types of leaves probably perform somewhat different functions during the season. Being spur-pruned, the trees would depend on the spur leaves to supply possibly a larger part of the nutrient requirements of both minerals and organic substances, including sugars, to the developing fruits; on the other hand the leaves of the leaders would be required to supply sugars, in particular, to the spurs and to the new leaves and wood of the new leader growth. Possibly therefore changes in chemical composition of the spur leaves might be related more closely to development of the fruit with concomitant alterations in nutrient and dry-matter status than those in the leader leaves. But, as the complex of leaves and fruit is a dynamic system, changes in composition of the leaves in respect of sugars may only occur when the demands of fruit, leaves, and twigs outpace or lag behind the supply of these compounds by the leaves whether spur or leader.

If the data of Tables 6 and 8 are regarded from this viewpoint it is seen that there is a relative constancy of level of glucose in both leaves and fruit; moreover the amount of glucose in the dry matter is of the same order in both types of material. In the 1958-59 season low sucrose, but high fructose, levels are shown in both spur and leader leaves at the time of rapid accumulation of fructose in the fruit; and, when sucrose begins to build up in the fruit, sucrose in both kinds of leaves rises and fructose falls. During the accumulation period in the fruit in 1958 both fructose and sucrose were low in the spur leaves. Apparently therefore the demands of the fruits are reflected in the levels of sugars in the leaves.

Accretion of Dry Matter, etc., in Fruits

From the data already presented, the increases in amounts of dry matter (including sugars), mineral elements, and nitrogen built into the fruits can be calculated for both seasons. In the early stages, when the fruits are small, the amounts of minerals and nitrogen per fruit are

TABLE 9. Accretion of Minerals and Nitrogen by Cox's Orange Fruits During Growth 1958-59 Season

(mg per fruit)

Date of Sampling	Days from Start of Sampling	Total Ash	Ca	Mg	P	K	Na	N
10.11.58	0	33.3	1.59	0.84	1.20	9.83	0.00	11.25
20.11.58	10	52.0	2.30	1.40	2.10	18.98	0.20	19.48
28.11.58	18	68.8	2.68	1.84	2.68	24.96	0.17	25.63
8.12.58	29	109.7	3.02	2.42	3.32	35.36	0.30	35.06
19.12.58	40	100.1	2.65	2.21	3.09	33.96	0.44	33.96
19. 1.59	71	243.7	3.8	4.8	3.8	99.0	1.90	74.3
18. 3.59	129	449.1	4.8	7.9	11.0	155.3	3.34	124.2
2. 4.59	144	438.7	5.0	7.9	12.4	166.9	3.81	102.5

minute, but with the rapid growth that occurs during December and January manufacture of organic material and intake of nutrients become appreciable. However only the data for the 1958-59 season will be considered in detail. Selections of the calculated data are given in Tables 9 and 10.

Although the percentage of a given constituent has been seen above to fall as the fruits grow, increase in size causes the total amount of a constituent in the apple to increase, usually through to maturity. From Table 9 it is seen that in the small fruits, other than for ash content, the amounts of mineral elements and nitrogen amount to only a few milligrammes per fruit. Intake of nutrients is rapid so that in about four weeks from commencement of sampling the amounts of ash, minerals, and nitrogen have trebled. By the middle of March, intake of ash had increased 13 times, of magnesium and phosphorus nearly 10 times, of nitrogen 11 times, of potassium 16 times, of sodium 33 times, but of calcium only 3 times. It is also interesting to note how in the early stages there are equal intakes of calcium and phosphorus and of potassium and nitrogen, but later on phosphorus and potassium outstrip their early companions. A further point (to which attention has been directed in the dry-matter data) is that magnesium, which was less than calcium early in the season, later exceeds calcium. This means too that on the fresh basis magnesium is present in greater concentration than calcium. The data of Table 9 suggest that during full maturation on the tree (tree-ripening) there may be a loss of nitrogen. This phenomenon, although at an earlier stage in the season, occurred also in the 1957-58 season.

TABLE 10. ACCRETION OF DRY MATTER AND SUGARS BY COX'S ORANGE FRUIT DURING GROWTH, 1958-59 SEASON
(g per fruit)

Date of Sampling	Days from Start of Sampling	Dry Matter	Total Reducing Sugars	Glucose	Fructose	Sucrose
10.11.58	0	0.52	0.055	0.022	0.033	0.005
20.11.58	10	1.09	0.167	0.072	0.095	0.034
28.11.58	18	1.86	0.331	0.132	0.199	0.038
8.12.58	29	3.54	0.870	0.305	0.565	0.036
9.12.58	40	3.88	1.274	0.374	0.900	0.137
19. 1.59	71	14.09	3.484	0.666	2.818	1.438
18. 3.59	129	23.89	8.266	2.007	6.259	6.140
2. 4.59	144	23.84	6.389	2.289	4.100	4.983

Accretion of dry matter and sugars is given in Table 10. When sampling began, the dry matter per apple was only 0.52 g; this was doubled in 10 days, and again in the next 10 days. Over the last few weeks of the experiment the amount per apple was 23.8 g, nearly a

fifty-fold increase from the original figure. In the very small fruits glucose and fructose were present in nearly equal amounts, 22 mg and 33 mg respectively, but very soon fructose outstripped glucose until at 40 days the amounts were glucose 374 mg and fructose 900 mg. Over the same period sucrose increased from 5 mg to 137 mg. By 18 March (129 days) the glucose was 2.007 g, fructose 6.259 g, and sucrose 6.140 g, illustrating the large build-up of sucrose in relation to the two reducing sugars. Fifteen days later glucose was higher than before at 2.29 g, but fructose and sucrose had fallen back to 4.10 g and 4.98 g respectively. Total sugars at the end of the season comprise approximately half of the dry matter of the apple.

No data can be calculated for the constituents of the leaves on a per-leaf basis because no counts of leaves per sample were recorded.

Amount of Constituent on Fresh-weight Basis

The data for percentages of ash, minerals, and nitrogen in the dry matter of the developing fruit have already shown that the ash and potassium content decrease to about one-third of their original values, while the other constituents of Table 5 fall to about one-fourth or one-fifth. Large increases in the amounts of the several constituents have been shown to occur over the season. How do these increases affect the percentages of the constituents when expressed on the fresh basis? Representative data on this matter from the two seasons of the experiment are given in Table 11.

Although, with the exception of sodium, all the constituents started in the first season at higher levels than in the second, by the end of the sampling periods the percentages of the several constituents were

TABE 11. Percentage of Constituents on the Fresh Basis of Cox's Orange Fruits

Season	Date of Sampling	Days from Start	Ash	Ca	Mg	P	K	Na	N
1957-58	14.11.57	0	0.871	0.037	0.026	0.041	0.300	0.002	0.381
	5.12.57	21	0.458	0.019	0.014	0.020	0.157	0.001	0.197
	22. 1.58	69	0.370	0.007	0.008	0.011	0.155	0.003	0.105
	19. 2.58	87	0.277	0.004	0.006	0.008	0.096	0.002	0.087
	4. 3.58	110	0.311	0.003	0.006	0.008	0.110	0.003	0.084
1958-59	10.11.58	0	0.752	0.036	0.019	0.027	0.222	0.002	0.254
	28.11.58	18	0.411	0.016	0.011	0.016	0.149	0.001	0.153
	29.12.58	50	0.263	0.007	0.006	0.007	0.080	0.001	0.102
	7. 1.59	59	0.257	0.005	0.005	0.006	0.110	0.001	0.084
	18. 3.59	129	0.278	0.003	0.005	0.007	0.096	0.002	0.077
	2. 4.59	144	0.283	0.003	0.005	0.008	0.108	0.002	0.066

essentially the same in both seasons. It is noticable however that magnesium was greater proportionately to calcium in the first than in the second, and also that nitrogen was somewhat higher in the first season. Such differences could conceivably affect the storage quality of the fruit and susceptibility to bitter pit.

Data similar to the above can be worked out for the percentages of sugars. Selected data are in Table 12.

TABLE 12. PERCENTAGES OF SUGARS IN FRESH MATERIAL OF COX'S ORANGE FRUITS

Season	Date of Sampling	Days from Start	Total Reducing Sugars	Glucose	Fructose	Sucrose
1957-58	14.11.57	0	1.12	0.63	0.49	0.33
	5.12.57	21	1.46	0.67	0.79	0.00
	22. 1.58	69	1.45	0.70	1.75	0.25
	19. 2.58	87	2.88	0.65	2.23	1.35
	4. 3.58	110	4.48	1.14	3.34	3.21
1958-59	10.11.58	0	1.24	0.49	0.75	0.12
	28.11.58	18	1.98	0.79	1.19	0.23
	29.12.58	50	3.03	0.82	2.21	0.41
	7. 1.59	59	3.70	0.74	2.96	1.19
	18. 3.59	129	5.12	1.24	3.88	3.80
	2. 4.59	144	4.13	1.48	2.65	3.22

Early in the season the percentages of sugars are low and remain so for glucose and sucrose, while fructose rises steadily with growth of the fruit. Later on, although glucose still remains almost constant in concentration in the fruit, both sucrose and fructose rise sharply, especially the latter. Rapid rises in these two sugars are associated with approaching maturity of the fruit. It is easy to see how only a few days difference in date of harvesting of the fruit will make a great difference to the concentration of sucrose and fructose; only at the end of the season when the fruit is past a suitable stage of maturity for cool storage does the glucose level rise, and then not to anything like the degree that sucrose and fructose do. It is of course known that starch content varies during growth but is present only in small amount at the usual harvesting stage.

The data already presented have shown that on the fresh basis most constituents decrease with growth and are present in low concentrations in the fruit at maturity; on the other hand in the fresh leaves there are increases in the concentrations of ash, calcium, and magnesium, relatively constant values for phosphorus and sodium, and

TABLE 13. Percentages of Minerals and Nitrogen in Leaves
(fresh basis)

Kind of Leaf	Date of Sampling	Days from Start	Ash	Ca	Mg	P	K	Na	N
Spur	10.11.58	0	2.36	0.318	0.068	0.065	0.84	0.010	0.030
	28.11.58	18	2.70	0.508	0.091	0.063	0.74	0.010	0.966
	29.12.58	40	2.64	0.508	0.110	0.048	0.67	0.008	0.830
	19. 1.59	71	3.08	0.620	0.107	0.052	0.77	0.009	0.904
	18. 2.59	101	3.69	0.803	0.132	0.064	0.60	0.008	0.884
	4. 3.59	115	3.47	0.682	0.123	0.046	0.46	0.008	0.712
Leader	10.11.58	0	1.95	0.323	0.061	0.053	0.60	0.007	0.833
	28.11.58	18	3.05	0.512	0.092	0.076	0.90	0.010	1.126
	29.12.58	40	2.73	0.491	0.094	0.054	0.69	0.007	0.953
	19. 1.59	71	3.37	0.713	0.126	0.061	0.76	0.010	1.026
	18. 2.59	101	3.53	0.696	0.117	0.065	0.58	0.008	0.937
	4. 3.59	115	3.24	0.616	0.105	0.053	0.49	0.008	0.838

decreases for potassium and nitrogen. Representative data for the spur and leader leaves of the 1958-59 season are given in Table 13.

If the data of Table 13 are compared with those of Table 11 a striking difference between the compositions of fruit and leaves will be seen; the latter are much the richer in all the constituents estimated. Another way of looking at the changes that take place in fruit and leaf during the season is to calculate the ratio of a given constituent in the leaf to that in the fruit. Table 14 gives data for the ratios leaf : fruit for calcium, magnesium, potassium, phosphorus, and nitrogen for spur and leader leaves and fruit of the 1958-59 season. Selected data are given for reasons of space.

A study of the above data shows that in general the values of the various ratios for a corresponding date and element are similar in both spur and leader leaves. The relative demands for phosphorus and nitrogen are alike over a good part of the growing season, and are much greater than those of the fruit (that is, the ratios have high values). On the other hand potassium shows relatively low values for the ratio of concentration of the element in leaf and fruit, the values varying between approximately 3 and 8. When the magnesium and calcium data are examined a state of affairs quite different from those with potassium, phosphorus, and nitrogen is found, with relatively low values of the ratio at the beginning of the season, but these rapidly increase to reach very high levels especially about the time when the fruit is reaching commercial maturity. At this time then competition between leaves and fruit for calcium seems to be intense.

TABLE 14. RATIOS OF CONCENTRATIONS OF CONSTITUENTS IN LEAVES AND FRUITS OF THE 1958-59 SEASON IN FRESH MATERIAL

Kind of Leaf	Date of Sampling	Days from Start	Ca leaf / fruit	Mg leaf / fruit	K leaf / fruit	P leaf / fruit	N leaf / fruit
Spur	10.11.58	0	9	3.6	3.8	2.4	4.1
	28.11.58	18	33	8.4	5.0	4.1	6.3
	29.12.58	40	76	20	8.4	6.6	8.1
	19. 1.59	71	141	23	7.4	12	12
	18. 2.59	102	201	26	6.5	12	15
	4. 3.59	115	184	25	4.5	6.5	12
Leader	10.11.58	0	9	3.2	2.7	2.0	3.3
	28.11.58	18	33	8.5	6.0	4.9	7.4
	29.12.58	40	73	17	8.6	7.4	9.3
	19. 1.59	71	162	27	7.3	14	13
	18. 2.59	101	174	23	6.3	13	16
	4. 3.59	115	166	21	4.8	7.5	14

DISCUSSION

The data of this paper show that the developing fruits of Cox's Orange undergo very great changes in chemical composition, with decreases in mineral concentration and increases in sugars as they approach maturity. It has been seen too that the proportions of, say, calcium and magnesium vary, in the early stages the former being present in the larger amount, and in the later stages the latter is the greater. Therefore when the fruit is picked one element or the other will be present in the greater amount. If one subscribes to the idea that the ratio of, say, calcium to magnesium in the tissue is important in governing whether bitter pit will appear at all, or in mild or severe form, then obviously the stage of maturity at picking will be important. Moreover it is not known whether levels of these elements, or the same ratios between them, that are satisfactory for Cox's Orange will be satisfactory for other varieties. This point will be examined in a later paper. Moreover since the nitrogen content, or at least the protein moiety of it, and the amounts of sugars present will influence rate of respiration, their levels in the tissues must be taken into consideration when discussing the storage quality of the fruit. The relationships between sugars contents and size of fruit and liability to pitting will also be considered later.

The present paper therefore gives the fundamental data for Cox's Orange grown in a certain orchard in the Nelson district. Data have been presented which indicate that seasonal effects may also be considerable on the chemical composition of the fruit. Whether differences

in chemical data and differences in incidence of bitter pit can be correlated satisfactorily will be discussed when data obtained in the spray trials are presented.

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THE ENDOPHYTIC FUNGUS OF PERENNIAL RYEGRASS

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Summary

In 1958 a survey was made of the incidence of the endophyte transmitted in certified perennial ryegrass seed (*Lolium perenne*) grown in New Zealand. Most lines of seed with high endophytic infection were strains harvested from long-established pastures. There was a lower percentage of endophyte in certified perennial ryegrass lines of seed grown from pedigree-bred strains of seed, and this appeared to be the result of loss of viability of the endophyte during seed storage in the early stage of multiplication of the seed. There was no endophytic infection in the seeds of short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*).

The endophytic hyphae were abundant in the nucellar tissue of the ovary, and, with the development of the endosperm, the hyphae became restricted to the layer of crushed nucellus round the periphery of the aleurone cells in the mature caryopsis.

Cultures of the endophyte grew best on ryecorn (*Secale cereale*) forming microconidia but no other spore-bearing structures.

Introduction

The study of the endophytic fungus in the seeds of perennial ryegrass (*Lolium perenne*) and short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*) was made as part of the investigation into the cause of facial eczema, a serious disease of sheep in New Zealand.

There are numerous and conflicting reports on the occurrence and identification of the endophytic fungus in ryegrass. This is mainly due to lack of any record of a fruiting stage to the difficulty of isolating and growing the fungus, and to the presence of blind-seed disease (*Gloeotinia temulenta* (Prill. et Delacr.) Wilson, Noble, et Gray) in the tissues of the ryegrass seed.

Endophyte Survey

In a previous survey of New Zealand-grown ryegrass seed, Neill (1940) showed that the endophytic fungus was normally present in certified perennial ryegrass, and absent from certified Italian ryegrass seed. With commercial lines of ryegrass seed harvested in the South Island, Neill reported that most of the seed was free from the endophyte.

In 1958, machine-cleaned lines of seed of certified perennial ryegrass and short-rotation ryegrass grown in the major ryegrass seed-growing areas, were used for an endophyte survey. The age, area of growing and history of the seed were known through departmental

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certification records. Samples were tested soon after harvest, as it was reported by Neill (1940) that the fungus tended to die in seed stored for more than a year.

Each sample of seed was mixed and mechanically divided until the sub-sample was reduced to approximately fifty seeds. These seeds were sown in a mixture of Vermiculite and non-sterilised soil in pots, and grown in a glasshouse for approximately 4 weeks. Neill (1941) reported that there was no transmission of the endophyte between infected and non-infected plants grown in the same box for 9 months. Attempts were made to grow the seedlings in sterilised soil, but this was soon abandoned when saprophytic-fungal contaminants grew on the soil and seedlings, masking the endophytic hyphae during examination of the plant tissues.

Ten seedlings were examined from each pot. Initially Neill's (1940) technique of examination for endophytic hyphae was used, mounting a small part of the basal leaf tissue of a seedling on a slide and staining with an aqueous solution of aniline-blue. With this method, the chloroplasts in the leaf cells masked the intercellular hyphae, and it was necessary to examine the cut ends of the leaf tissue to detect the fungus. A quicker method was to boil short lengths of excised seedling stems for five minutes in a dilute aniline-blue staining solution, as recommended by Popp (1951) for the detection of loose-smut hyphae in the embryonic tissue of wheat and barley seed. The leaf cells were partially cleared, the plant tissue staining a light blue and the endophytic hyphae a deeper blue. The endophyte in leaf tissue has been described by Neill (1940), the major diagnostic features being the long parallel intercellular hyphae with non-staining septa, and the occasional right-angled branching which joins two adjacent hyphae.

Table 1 shows the results of the endophyte survey. There is a significant difference between lines of certified Permanent Pasture class of seed grown in the Hawke's Bay/Poverty Bay area, and those grown

TABLE 1. Incidence of Endophyte in Perennial Ryegrass Seed

Certification Class of Seed	No. of Lines Examined	Seed-growing Area	% Plants with Endophyte
Pedigree	17	Hawke's Bay	11.2
Pedigree	20	Canterbury	18.0
Mother	19	H. Bay, Poverty Bay, Wellington	13.1
Mother	20	Canterbury, Otago	24.5
Permanent Pasture	19	Hawke's Bay	62.6
Permanent Pasture	11	Poverty Bay	62.7
Permanent Pasture	32	Canterbury	28.1

in the Canterbury area. There is also a significant difference between lines of Hawke's Bay/Poverty Bay-grown seed certified in the Permanent Pasture class, and those lines of seed certified in other classes.

The survey was repeated, using certified short-rotation ryegrass seed. Of all the seedlings examined, only two were infected with the endophyte. It is possible that these two seeds were derived from perennial ryegrass plants that had invaded or been introduced into short-rotation-ryegrass pastures harvested for seed. It was further shown that there was no endophyte in any of twenty samples of seed harvested from single crosses of short-rotation ryegrass plants grown at Grasslands Division, DSIR, as part of their progeny-testing programme for the selection of pedigree-bred strains of seed.

ENDOPHYTE IN SEED OF LONG-ESTABLISHED PERENNIAL RYEGRASS PASTURES

In the Government seed-certification scheme for perennial ryegrass, the initial pedigree-bred strain of seed is multiplied, and each increase of the seed classifies it in a separate class. Certified Permanent Pasture perennial ryegrass seed differs from all other classes of certified seed, in that it may be harvested from either a pedigree-bred or a non-pedigree-bred strain of perennial ryegrass.

The highest percentage of endophyte was in the certified Permanent Pasture class of seed grown in Hawke's Bay and Poverty Bay. In these districts there still exist areas of perennial ryegrass pastures originally sown by the early settlers. Some of these long-established strains of perennial ryegrass pastures are regularly harvested for seed crops, and the seed is certified in the Permanent Pasture class.

In 1958, Instructors of the Department of Agriculture collected information from the growers of those lines of certified Permanent Pasture perennial ryegrass seed which showed 70% or more endophytic infection, there being no high-infection lines in any of the other classes of certified seed. Where the age of the pasture was more than twenty years old it was classified as long established. Of the 17 lines of seed with 70% or more endophytic infection, 16 were harvested from long-established pastures, or pastures recently sown with seed harvested from such areas.

In the Canterbury district of the South Island there are also areas of long-established strains of perennial-ryegrass pastures but these are not usually harvested for seed as is the practice in Hawke's Bay and Poverty Bay. It is believed that lines of certified Permanent Pasture seed grown in the Canterbury district are mostly pedigree-bred strains of seed, and therefore comparable with lines of seed certified in the Pedigree and Mother classes.

TRANSMISSION OF THE ENDOPHYTE IN THE SEED

At present the only known method for spread of the endophyte is by seed or plant vegetative propagation. Sampson (1935) concluded

that the endophyte was mechanically inherited from the female parent only, although Neill (1940) considered that sometimes a genetic factor for resistance may prevent inheritance of the fungus.

Freeman (1903) made a detailed study of the endophyte of darnel (*Lolium temulentum*). This work confirmed previous reports that the darnel endophyte forms a hyphal layer outside the aleurone cells in the crushed nucellus of the seed. The first comprehensive description of an endophytic fungus in perennial ryegrass was by McLennan (1920). This work was completed before the published description of the blind-seed disease of ryegrass (*Gloeotinia temulenta*) by Neill and Hyde (1939, 1942), and the fungus described by McLennan appears to be a description of the hyphae of both the endophyte and blind-seed. Sampson (1937) reported the presence of two endophytes of perennial ryegrass which for convenience were named the first and second endophyte of *Lolium perenne*. The mycelium of these two endophytes was distinguished by the intensity of staining, and growth of the fungi on artificial medium. Neill (1940) examined mature seeds of ryegrass and reported the endophytic hyphae in the crushed nucellar tissue overlying the aleurone layer, but not penetrating these cells. There were scant endophytic hyphae in the embryonic tissue, but no hyphae were observed in the endosperm, a feature distinguishing the endophyte from the blind-seed fungus.

In 1958, flowering stems of perennial ryegrass were collected from a long-established pasture in the Wanganui district. To ensure that the material was free from blind-seed disease, microscopic examinations

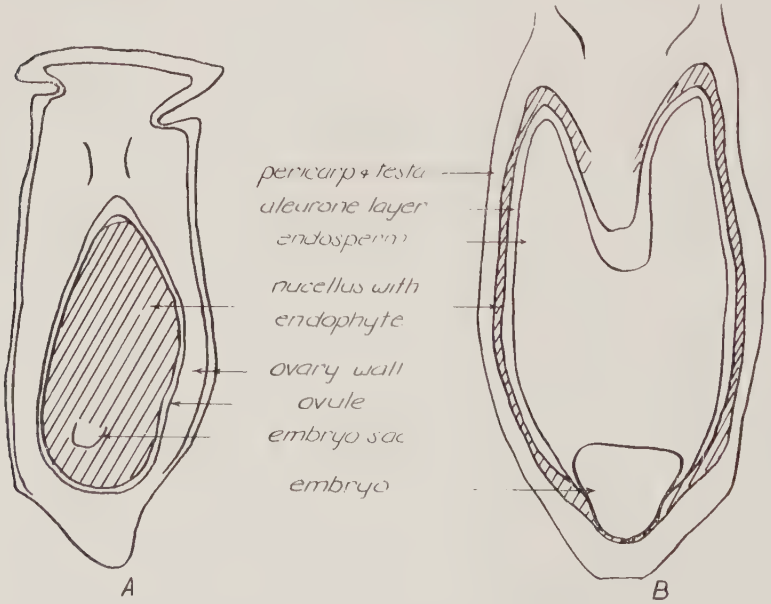

Fig. 1.—Longitudinal section of a perennial ryegrass ovary (A), and a mature caryopsis (B). The hatched areas represent the nucellar tissue with endophytic hyphae.

were made of washings from the seeds, no blind-seed macroconidia being observed. Fragments of the pith were scraped out of each flowering stem and examined for endophyte, following the technique described by Sampson (1935). When the endophytic hyphae were found in the pith of the flowering stem, the ovaries and caryopses were removed and the sections prepared with a microtome. Several stains were tried, the most successful being crystal violet with a counterstain.

Fig. 1A is a longitudinal section of an ovary of perennial ryegrass near the time of fertilisation. The endophytic hyphae are abundant throughout the nucellar tissue of the ovule.

Fig. 1B is a longitudinal section of a mature caryopsis. The development of the endosperm has caused a crushing of the nucellar tissue to a thin layer round the periphery of the aleurone cells, and the endophyte is in this zone of nucellar tissue.

Fig. 2.—The endophytic hyphae in the nucellus of the ovary (A), and overlying the aleurone cells in a mature caryopsis (B).

Fig. 2A shows the endophytic hyphae in the nucellus of the ovary. The hyphae are abundant, following the contours of the nucellar cells and joining adjacent hyphae between the junctions of opposite cells. The hyphae are intercellular, approximately 1μ in diameter, deep staining, and with the characteristic non-staining septa.

Fig. 2B shows the endophytic hyphae in the remnants of the nucellus overlying the aleurone cells in a mature caryopsis. The hyphae are 1–2μ in diameter, slightly contorted, branched, intercellular, unevenly stained, and septate.

Fig. 3 is part of the zone of hyphae in the nucellus of a mature caryopsis. The section has been cut obliquely, resulting in an extension of the nucellar area between the aleurone cells and the testa.

GROWTH OF THE ENDOPHYTE IN CULTURE

Sampson (1937) grew the first endophyte of *Lolium perenne* on egg medium. Neill (1940) was not able to repeat this work, but isolated and grew the endophyte from infected seedlings immersed in a nutrient solution, where the endophyte was in partially anaerobic conditions. Using Neill's technique, the fungus was isolated and transferred to various natural media in milk bottles. A plastic pot-cleaner

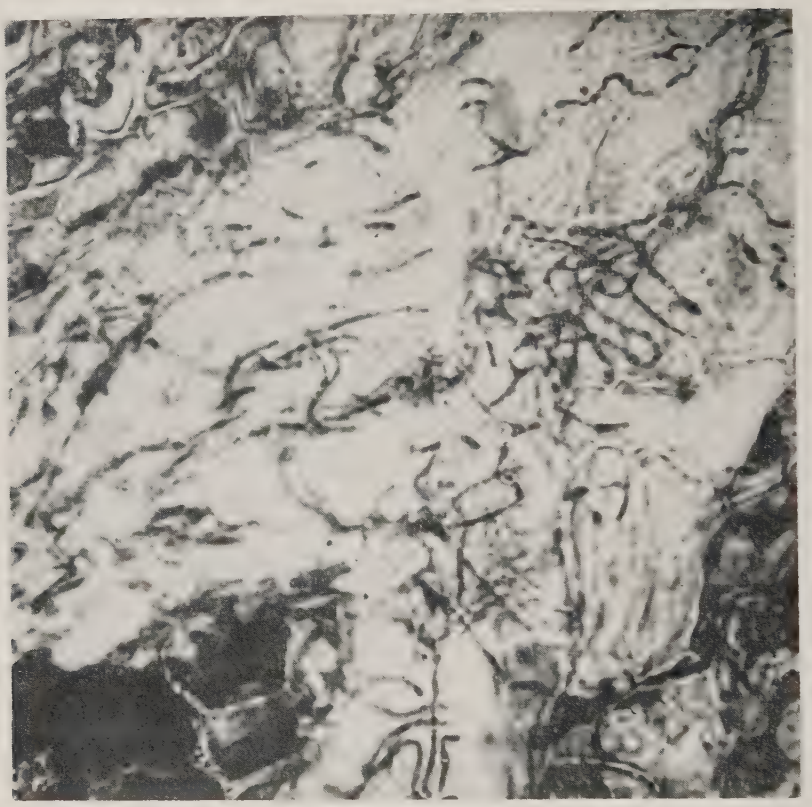

Fig. 3.—The zone of hyphae in the nucellus of a mature caryopsis. The light area is the nucellus with the dark-stained endophytic hyphae, and the dark cells in the bottom left corner are the aleurone cells.

was placed at the bottom of each milk bottle and water added to prevent drying of the medium during the long period of incubation. The medium was moistened with the addition of a small quantity of peptone-glucose solution and put on top of the pot-cleaner. The bottles were autoclaved, inoculated, and incubated for 8 weeks at 23°c and subsequently placed in a 5°c refrigerator and examined periodically for fungal fructifications. This work was repeated, growing the fungus in a wide range of temperatures and light. No fructifications were observed other than the microconidial sporodochia previously reported by Neill (1941). Abundant mycelial growth occurred on the whole seed of ryecorn (*Secale cereale*) and barley. There was weak growth of the fungus on potato and carrot cubes and ryegrass seed, and no growth on ryegrass leaves.

Discussion

The present survey shows that the incidence of endophyte in certified perennial ryegrass seed is less than reported by Neill in 1940. Since Neill's work, the Government seed certification scheme for peren-

nial ryegrass seed has been based on a plant-breeding programme, using pedigree-bred strains of seed to replace the strains harvested from long-established pastures in the Hawke's Bay and Poverty Bay districts. Seed from these long-established pastures is now certified as Permanent Pasture class and the highest incidence of endophyte is recorded in these lines of seed.

The Pedigree and Mother class and probably the Permanent Pasture class of seed harvested in the Canterbury district are grown from pedigree-bred strains of seed selected at Grasslands Division, DSIR, as part of the plant-breeding programme. In this plant-breeding work, several selected perennial ryegrass plants are allowed to inter-pollinate and the resulting seed harvested and classified as nucleus isolation seed. These nucleus isolations are made periodically, there usually being several years between each isolation. A proportion of this seed is used each year for seed increase and subsequent inclusion in the appropriate seed certification class. Some of the nucleus isolation seed will therefore be several years old before it is released for seed increase. Neill (1940) reported that the endophyte remains viable up to approximately 18 months in stored seed. The lower percentage of endophyte in certified perennial ryegrass lines of seed grown from pedigree-bred strains appears to be the result of withholding part of the nucleus isolation seed for varying periods of time. In the certified Permanent Pasture class of seed grown in the Hawke's Bay and Poverty Bay districts, the high incidence of endophyte is associated with the lines of seed harvested from long-established strains of perennial-ryegrass pastures.

The endophyte is transmitted in the crushed nucellar tissue of the seed. The fungus does not appear to affect the seed germination, there being no obvious association between germination and endophyte counts of the seed. Neill (1952) found no significant difference between weights of herbage, or preference in animal grazing, between endophyte-infected and endophyte-free plants of perennial ryegrass. Feeding trials on birds, rodents and sheep, using cultures of the endophyte and infected seed, showed no evidence of toxicity to the animals (Cunningham 1958).

At present, the known methods of transmission of the endophyte are by seed or plant vegetative propagation. If dissemination of the fungus was only by these methods, an endophyte-free seed would be the parent of endophyte-free progeny, and there would be no reinfection of these plants. In a recent publication of the sexual reproduction of blind-seed disease of ryegrass (*Gloeotinia temulenta*) Griffiths (1958) described the fertilisation by microconidia of compatible strains of the blind-seed fungus. The endophyte may also be heterothallic, the microconidia functioning as male gametes in fertilisation and the subsequent development of spore-bearing fructifications. The dissemination of the endophyte requires further investigations.

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WINTER GROWTH OF LUCERNE (*Medicago sativa*)

I. VARIETY PERFORMANCE IN NEW ZEALAND

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(*Received for publication, 31 August 1959*)

Summary

A number of lucerne varieties covering a wide range of winter activity was grown at five stations in New Zealand. By local standards the winter climate at the five stations would vary from mild to severe. The relative winter growth of these lucernes was assessed by measuring stem length in mid winter and early spring.

The performance of varieties was similar at the different stations, in different years, and in winter and spring. The performance of individual plants was also similar in different seasons and years. From this it is inferred that one suitable strain would be capable of giving high winter production over all the present area in which lucerne is grown.

The varieties in order of decreasing winter growth were: African, Hunter River, Provence; Marlborough, Du Puits; N.Z. Certified, Parachinar; Rhizoma. These results are quite different from those obtained by Zaleski at Cambridge, England, and this suggests that different environmental factors affect winter growth there and in New Zealand.

There was a close correlation between stem length and production within the three varieties in which this relationship was determined.

Introduction

Early growth in spring is a valuable attribute in any pasture plant. This is especially true when growth is curtailed by drought in late spring or early summer, as it is in New Zealand, where the soils on which lucerne is grown extensively are shallow and overlie deep gravel beds. They hold little available moisture, and in most seasons lack of moisture limits growth from early November to March (Rickard unpubl.). In some years the summer drought may start earlier than this, or continue longer, and there are extensive areas where it does so regularly.

From April or May to September or October, moisture is usually adequate for growth, but active growth of lucerne is limited during this period by winter conditions. However, some "varieties" or regional strains of lucerne grow more than others during this period, so it is of some importance to know which ones are most winter active.

Zaleski (1954), at Cambridge, England, measured the height of the longest stem of several lucerne varieties at the end of April, and classified them as follows:

Early—Eynsford Strain, Du Puits, Chartainvilliers, W268.

Mid Season—Provence, Strain B, Marlborough.

Late—Hunter River, Eynsford No. 1, Saladina.

Extra Late—Rhizoma, Grimm.

Some of these varieties have been grown at Lincoln for a number of years, and it was apparent that the order of winter growth at Lincoln was not the same as at Cambridge. In fact, apart from the extra-late varieties, Rhizoma and Grimm, the order was almost the reverse of that at Cambridge.

In order to investigate this situation more thoroughly and to see if the results at Lincoln were typical of those in the main lucerne-growing areas of New Zealand, the experiments reported here were conducted.

Material and Methods

The varieties used were Hunter River, Rhizoma, Du Puits, Marlborough, Provence, N.Z. Certified (Strain B), African, and Parachinar. These varieties cover a wide range of winter activity. N.Z. Certified and Provence were grown from certified seed. Parachinar was obtained from Peshawar, north-west Pakistan, and the other varieties were from commercial seed known to be typical of the variety. Only one seed line of each variety was used in all of the trials.

Some of these varieties were grown at Palmerston North, Lincoln, Ellesmere Spit, Omarama, and Gore. Details of the trials are shown in Table 1, together with minimum and maximum temperatures for June 1956 at each station. No temperature records were available for Ellesmere Spit, but minimum temperatures would be higher there than at Lincoln, but probably lower than at Palmerston North.

TABLE 1. Composition and Arrangement of Trials

Varieties	Gore	Palmerston North	Lincoln	Omarama	Ellesmere Spit
Hunter River	+	+	+	+	+
Provence	+	+	+		+
Du Puits	+	+	+	+	+
N.Z. Certified	+	+	+	+	+
Rhizoma	+	+	+	+	+
Marlborough	+	+	+	+	+
African			+	+	+
Parachinar			+	+	+
Replications	8	8	8	5	3
Plants per plot	16	14	19	22	13
Mean Min. June 1956 °C	1.3	5.6	0.3	—1.3	
Mean Max. June 1956 °C	10.0	13.3	11.8	9.9	

All trials were arranged as randomised blocks. Each plot consisted of one row of plants, 75 cm apart. Rows were also 75 cm apart. The trials were planted at Palmerston North and Gore in the spring of 1955, at Ellesmere Spit in the autumn of 1956, and at Lincoln and Omarama in spring of 1956.

The plants were cut to within a few centimetres of their crowns in early April (autumn), and in mid June the length of the longest

TABLE 2. MEAN LENGTHS (cm) OF LONGEST STEMS OF LUCERNE VARIETIES

Variety	Gore				Palmerston North				Ellesmere Spit		Lincoln			Omarama
	1956		1957		1956		1957		1956	1957	1957		1958	1957
	6 July	6 Sept.	19 June	26 Sept.	5 June	12 Sept.	5 July*	23 Sept.	22 Sept.	5 June	24 June	23 Sept.	10 Sept.	29 Sept.
African									16.1	33.3	37.8	35.5	23.9	20.4
Hunter River	29.2	15.7	46.6	29.9	55.3	36.9	24.3	35.7	11.6	26.3	33.8	33.6	20.9	18.0
Provence	21.4	14.7	41.4	32.9	45.7	34.5	17.3	36.5	11.9	23.4	27.3	35.7	22.5	
Marlborough	22.4	14.4	41.5	29.1	46.9	30.4	16.1	33.2	9.2	20.4	27.8	32.2	21.1	16.4
Du Puits	20.3	13.1	42.3	30.0	49.0	28.5	14.9	27.2	9.4	22.0	24.9	32.0	18.5	18.8
N.Z. Certified	17.3	10.7	32.5	23.9	39.1	20.4	10.7	19.9	7.6	19.1	23.1	26.8	16.1	14.8
Parachinar									7.7		21.0	27.5	17.1	14.6
Rhizoma	—	—	23.9	15.5	36.0	14.6	—	17.5	6.4	—	17.0	18.5	10.2	12.6
L.S.D. 5%	4.2	1.4	3.0	2.2	2.8	3.6	4.7	4.3	1.8	2.0	2.7	2.2	2.2	2.0

* Cut on 3 May.
— Almost completely dormant.

stem on each plant was recorded. The plants were then cut again, and measured once more in September. Stem elongation between April and mid June is termed early winter growth, and that between mid June and September late winter growth. Elongation over the whole period is called winter growth.

Plot means were used to calculate variety means, and in computing the significance of variety differences. Plants at Lincoln were also measured on 28 August and 10 and 23 September in 1957, and on 7, 13, 21 and 28 August and 4 and 10 September in 1958 to determine their growth rates.

Results

Variety Means

Mean stem lengths of the varieties at the different stations and seasons are presented in Table 2.

Though the varietal order differed slightly from station to station and from year to year, the general order in early winter was African with the longest stems, followed by Hunter River; Provence; Marlborough, and Du Puits; N.Z. Certified, Parachinar; Rhizoma. In late-winter growth Provence and Hunter River were about equal, with Provence perhaps a little better than Hunter River. Du Puits and Marlborough were also about equal, followed by N.Z. Certified and Parachinar, with Rhizoma well below the rest. African grew more in the early winter at Ellesmere Spit and Lincoln than any other variety. In the late winter, it was better than any other variety at Ellesmere Spit, and was equalled only by Provence and Hunter River at Lincoln, and by Du Puits at Omarama. Parachinar was throughout similar to N.Z. Certified.

TABLE 3. Within-variety Correlation Coefficients for Growth in Early and Late Winter and in 1956 and 1957

Variety	Gore				Palmerston North				Lincoln
	EW	LW	1956	1957	LW	EW	1956	1957	LW:EW
	1956	1957	LW	EW	1956	1957	LW	EW	1957
African									.60**
Hunter River	.45**	.53**	.62**	.55**	.43**	.40**	.35*	.40**	.43**
Provence	.50**	.64**	.42**	.48**	.58**	.37**	.41**	.63**	.45**
Marlborough	.56**	.59**	.59**	.47**	.34**	.40**	.58**	.38**	.47**
Du Puits	.49**	.48**	.53**	.42**	.23	.36**	.49**	.05	.53**
N.Z. Certified	.64**	.57**	.57**	.47**	.46**	.42**	.64**	.34**	.44**
Parachinar									.16
Rhizoma		.42**	.61**		.45**		.58**		.34**

EW Early Winter
LW Late Winter

Correlations between Seasons and Years

Within-variety correlations for early-winter and late-winter growth, and for growth in 1956 and 1957, are presented in Table 3.

These correlation coefficients were, with three exceptions, positive and significant, showing that in general those plants which grew most in the early winter grew most during the late winter in both years.

Growth Rates

Observations on these and other plots suggested that the recorded differences between varieties arose because some varieties commenced growing earlier and finished later, rather than that they grew at different rates after starting growth.

At Lincoln in 1957 plants were measured on 28 August and 10 and 23 September, and in 1958 at approximately weekly intervals from 7 August to 10 September. Growth increments for both years are given in Table 4, and the growth rates for 1958 in graphical form in Fig. 1.

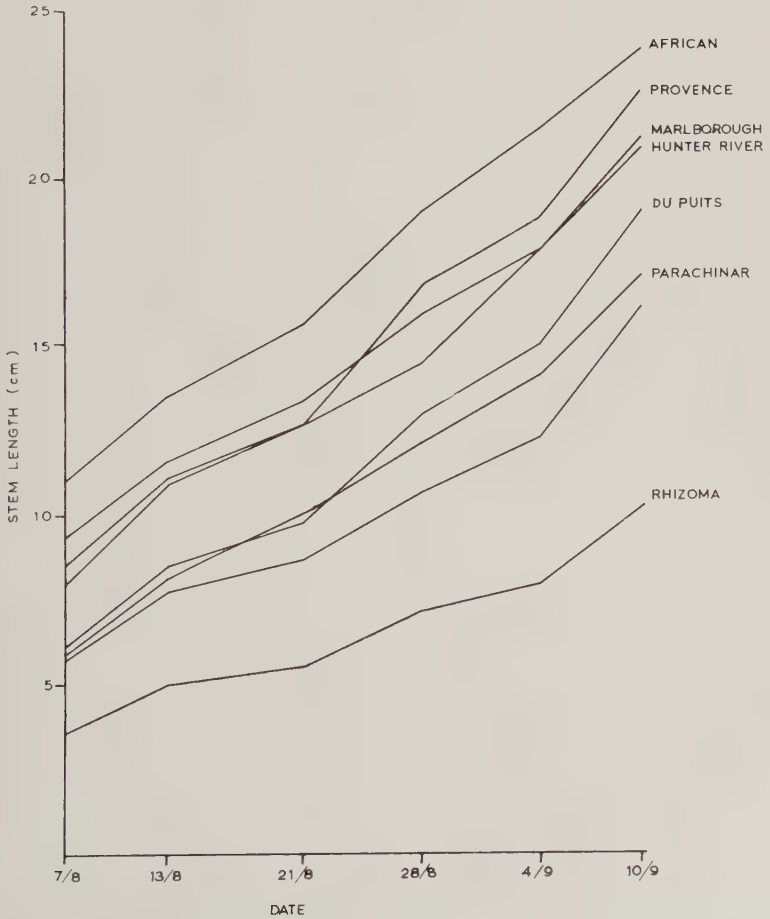

Fig. 1.—Growth of lucerne varieties, spring 1958.

TABLE 4. STEM LENGTH AND GROWTH INCREMENTS (cm) OF LUCERNE AT LINCOLN IN SPRING 1957 AND 1958

Variety	1957			1958		
	28 Aug.	23 Sep.	Incr.	7 Aug.	10 Sep.	Incr.
African	22.1	35.5	13.4	11.0	23.9	12.9
Hunter River	17.8	33.6	15.8	9.3	20.9	11.6
Provence	16.6	35.7	19.1	8.0	22.5	14.5
Marlborough	15.1	32.3	17.2	8.5	21.1	12.6
Du Puits	13.3	32.0	18.7	6.1	18.5	12.4
N.Z. Certified	10.6	26.8	16.2	5.8	16.1	10.3
Parachinar	11.9	27.5	15.6	5.9	17.1	11.2
Rhizoma	7.2	18.5	11.3	3.6	10.2	6.6
L.S.D. 5%	1.5	2.2	1.7		2.2	2.2

Growth rates for all varieties during these periods were not constant. In 1957, Provence and Du Puits grew significantly more than the other varieties apart from Marlborough, while Marlborough, N.Z. Certified, Hunter River, and Parachinar all grew about the same amount, and more than African, which in turn grew more than Rhizoma. In 1958 Provence grew more than all the other varieties, except African, while Rhizoma grew less than any other.

The results show that some varieties grew more rapidly than others, but that these differences in growth rate were not sufficient to account for the varietal differences at the end of the period; differences in length at the beginning of the measuring period were also important in determining the final varietal order. These data are not sufficient to show that one variety started growing before another. However, on 7 August, 15 of the 150 Rhizoma plants had no perceptible growing shoots, and these results can be interpreted as showing that differences in lengths of the winter-dormant period determine the differences on 7 August.

Relation between Stem Length and Production

Stem length is only one part of the components of fodder production from lucerne plants, the components being stem number and the mean weight of the stem and its leaves. Stem length is the easiest component to determine, and can be obtained without cutting the plants. At Lincoln in 1958 the relationships between stem number, stem length, and plant weight were examined for some thirty plants each of N.Z. Certified, Hunter River, and Provence. These varieties were chosen because of their contrasting stem lengths and stem numbers.

Mean stem lengths, numbers of stems, and weight per plant, and the correlations between these variables, are given in Table 5.

The mean weight of N.Z. Certified, the shortest variety, was significantly higher than that of Hunter River, but not significantly

TABLE 5. YIELD COMPONENTS AND THEIR RELATIONSHIPS, LINCOLN 1958

Variety	DF	Variety Means: Length (cm)	No. Stems	Weight (g)	Correlations: Length: Weight	No. Stems: Weight	No. Stems: Length
N.Z. Certified	27	17.5	166.4	219.6	.78**	.79**	.47*
Hunter River	29	23.4	74.2	162.5	.75**	.73**	.05
Provence	37	23.5	77.7	191.0	.75**	.77**	.50**
All Plants					.49**	.68**	.17

greater than Provence. N.Z. Certified produced many more stems than the other two. Within each variety, stem length and plant weight were closely correlated, so, for populations which did not vary too greatly in number of stems per plant, length could be used with discretion as an aid to selection for yield. Number of stems was also closely correlated with yield, but its determination requires more labour than measuring yield itself. In Provence and N.Z. Certified, number of stems and length were positively correlated, and this probably reflects general differences in plant vigour. In Hunter River they were not correlated, and in the mixed population the correlation was slightly but not significantly negative.

DISCUSSION

The general order of winter growth of the varieties in these trials was African; Hunter River and Provence; Marlborough and Du Puits; N.Z. Certified and Parachinar; Rhizoma. This order did not vary greatly from station to station, from year to year, or between early and late winter. There was a high positive within-variety correlation between one year and another, and between early and late winter.

All this evidence suggests that the relative winter growth of these varieties at the different stations and in different years and seasons was determined by reaction to similar environmental factors.

It seems likely that the results of these trials would be generally valid for all the lucerne-growing areas of New Zealand, as the trial stations covered as wide a range of winter severity as would be encountered throughout the districts where lucerne is grown. From this it may be concluded that one suitable variety would give high winter production wherever lucerne is normally grown.

From these and other trials which have been conducted at Lincoln and other parts of New Zealand (Palmer unpubl.) it can be concluded that varieties from the tropical regions, typified by African, are the most winter active here. Lucernes from the Mediterranean region, or regions with Mediterranean-type climates such as South Africa or California rank next, Provence perhaps a little later, and varieties from Northern France later still. Lucernes adapted to cold Continental climates, such as Rhizoma, Ladak, and Ranger, are the latest varieties that have been grown here. These reactions would be expected if winter dormancy is necessary for survival in areas with

cold winters, but is induced by some factor in addition to low temperature, such as day length, which varies regularly over the year. Tysdal (1933), Canode (1956), Morley *et al.* (1957), and Schonhorst *et al.* (1957) have shown that day length does affect the winter activity of lucerne varieties.

The results obtained in the trials reported here are similar to those reported by Morley *et al.* (1957) at Canberra, Australia, but quite different from those reported by Zaleski (1954) at Cambridge, England. Presumably, similar environmental variations have determined variety reactions in New Zealand and Canberra, but a different set at Cambridge. Differences in day length and temperature regimes could account for the observed effects.

Number of stems per plant probably makes a relatively greater contribution to yield in spaced plants, where the different stem-producing potentialities of the plants can express themselves, than it does in swards, where this potentiality is restricted. This aspect of production per unit area is at present being investigated. The interim results show that a variety such as N.Z. Certified, with its dense stem producing capacity, produces more stems per unit area under sward conditions than varieties that have few stems per plant, and in yield this compensates to a fair degree for its lack of stem length. This may be specially important in early spring production when light, rather than moisture, probably limits growth, and the varieties producing a greater number of stems may intercept more of the incident light than a more sparsely stemmed variety. However, in swards, early-growing varieties such as Provence or Hunter River produce more per unit area in the winter than N.Z. Certified, though this variety may produce as much as the others later in the spring. It would appear that a variety with the dense stem production of Strain B and the early growth of Hunter River or Provence would produce more than any varieties currently tested.

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EFFECT OF GIBBERELLIC ACID AND NITROGEN ON WINTER GROWTH OF PASTURE

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Summary

The effects and interaction of gibberellic acid and nitrogen on pasture production, composition, and nitrogen levels were studied on a 2-year-old sward of ryegrass and white clover following mid-April application when the natural growth rate was slow.

The application of gibberellic acid caused a rapid stimulation of growth, reaching a maximum within four weeks. Fertiliser nitrogen was slower acting, but its production equalled that obtained from gibberellic acid six weeks after application. Over the subsequent six months gibberellic acid depressed yields significantly, while slight nitrogen responses continued. No significant interactions were recorded.

Gibberellic acid caused yellowing of the herbage and stem elongation up to the first mowing, six weeks after application.

Yield responses and depressions of individual species resulting from treatment with gibberellic acid are presented. There was evidence of an increase in sward density within six weeks of application.

Gibberellic acid caused increased yields of nitrogen (oven-dry material) up to six weeks after application. Samples taken fourteen weeks after application showed a lowering of nitrogen yields from the acid treatment.

Introduction

In recent years a large number of overseas workers have reported the effects of gibberellic acid on various plant physiological processes such as the initiation of flowering (Lindstrom *et al.* 1957), reversal of dwarfism (Phinney 1956), increased fruit set (Wittwer *et al.* 1957), the breaking of dormancy of seeds (Kahn *et al.* 1957) and vegetative organs (Rappaport 1956), and finally stem elongation and increased yields (Brian *et al.* 1954).

Since New Zealand's economy is based on its pastoral industry, the field-scale pasture trials of Morgan and Mees (1958) are of particular interest. They showed that gibberellic acid was able to produce a grazeable growth of grass more rapidly than a nitrogen fertiliser in spring and autumn.

The trial reported was designed to verify broadly under New Zealand conditions the out-of-season pasture responses obtained in England by Morgan and Mees, and to compare the responses with those obtained from fertiliser nitrogen.

Experimental

The trial was commenced on 17 April 1958 on a second-year sward with short-rotation and perennial ryegrass dominant, white clover sub-dominant, and some cocksfoot, timothy, and red clover present.

The soil was a recent alluvial silt loam of high fertility with pH 6.6, Ca 17, K 16, and P 10 in the 0–3 in. layer.*

The trial design was limited in both replication and plot size by the small amount of gibberellic acid available. A three-replicate randomised-block layout was used with a plot size of 13 ft × 2 ft. The following treatments were compared:

1. Control.
2. Gibberellic acid 0.9 oz per acre.
3. Nitrolime (20.5% N) 2 cwt per acre.
4. Gibberellic acid 0.9 oz per acre + nitrolime 2 cwt per acre.

A basal dressing of 6 cwt per acre superphosphate was applied although this may not have been necessary in view of the work of Brian *et al.* (1954), who found that the effects of gibberellic acid were not influenced by the nutrient status of the solutions in which wheat plants were grown.

The gibberellic acid was applied with a motorised plot sprayer, the solution being prepared by dissolving the acid in ethyl alcohol and adding sufficient water + Agrol LN wetting agent to the solution to bring the rate of application up to 23.8 gallons per acre. Nitrogen fertiliser was applied by hand.

A few hours after application of the treatments light rain fell. This meant that portion of the gibberellic acid solution may have been washed off the plant leaves before much absorption could take place, and thereby reduced the effective rate of application of the acid.

Results in the early stages were measured by eye observation, followed by mowing and return of clippings (Lynch 1947) to provide yield data, herbage dissection of the mown samples and point analysis (Levy and Madden 1933) to measure pasture composition changes, and height point analysis (Spedding and Large 1957) to measure changes in height of growth. Total nitrogen levels of oven-dry material were determined.

Results

Visual Observations

A noticeable stimulation of growth accompanied by a yellowing of herbage was noted one week after application of gibberellic acid. Within two weeks the growth response was estimated as twice that of untreated plots, and marked chlorosis was evident. The colour change occurred in all species present, and was most marked on cocksfoot. Clovers showed slightly less yellowing than grasses. Stem elongation and narrowing of the leaf blade of grasses was evident, particularly in the case of perennial and short-rotation ryegrass, while clovers showed an increase in length of stem and leaf area as judged by eye observation. At this time there was no obvious response from nitrogen, other than a tendency towards the typical darker-green colouration.

* Parts Ca per 20,000 of soil extract
Parts K „ 200,000 „ „ „
Parts P „ 50,000,000 „ „ „

Over the subsequent two weeks there did not appear to be any further response to gibberellic acid, while nitrogen and nitrogen + gibberellic acid plots were showing a response estimated to be nearly equal to that obtained from the acid alone. Some recovery of the green colouration on acid-treated plots was apparent, and by July the regrowth of herbage on the plots cut the previous May showed no departure from the normal green colour of herbage on untreated plots.

Height Point Analysis

This was employed as a method of measuring stem elongation of the predominant species.

Results obtained five weeks after application are shown in Table 1.

TABLE 1. HEIGHT INDEX (in.)

	Ryegrass	White Clover
Control	2.9	1.5
Gibberellic acid	4.2	2.5

These results show an increase of 45% and 65% in the mean length of ryegrass and white clover stems respectively.

Fourteen weeks after application (eight weeks after mowing) recovery growth was again analysed. No difference in height of growth resulting from treatment with gibberellic acid was evident.

TABLE 2. TOTAL DRY-MATTER YIELDS (lb/acre)

	28.5.58	Diff.	31.7.58	Diff.	17.9.58	Diff.	7.10.58	Diff.	10.11.58	Diff.
Mean of:										
No G.A.[1]	1030		550		570		1020		1860	
G.A.	1150	120*	470	—80	380	—190**	860	—160**	1830	—30
No N[2]	1030		490		420		920		1820	
N	1150	120*	540	50	530	110**	950	30	1880	60
Individual treatments:										
Control	960		570		510		1020		1830	
G.A.	1110		400		330		820		1810	
N.	1110		520		620		1010		1900	
G.A. + N.	1190		550		430		890		1850	
C. of V.[3]	5.3%		18.3%		10.9%		7.9%		3.0%	

[1]G.A.—gibberellic acid.
[2]N—nitrogen.
[3]C. of V.—coefficient of variation.

* Significant at 5% level
** Significant at 1% level

TABLE 3. HERBAGE-DISSECTION DATA (lb/acre dry matter)

Mean of:	28.5.58			31.7.58			17.9.58			7.10.58			10.11.58		
	Yield	Diff.	% Diff.	Yield	Diff.	% Diff.	Yield	Diff.	% Diff.	Yield	Diff.	% Diff.	Yield	Diff.	% Diff.
Ryegrass															
No G.A.	800			440			410			610			990		
G.A.	870	70*	9	380	—60	—14	280	—130**	—32	590	—20	—3	1150	160	16
No N	800			390			310			560			1030		
N	870	70*	9	430	40	10	380	70*	23	640	80	14	1110	80	8
Sig. of Int.	N.S.			N.S.			N.S.			N.S.			N.S.		
C. of V.	5.1%			18.9%			13.6%			15.5%			15.5%		
Cocksfoot + Timothy															
No G.A.	100			50			70			90			270		
G.A.	120	20	20	40	—10*	—20	50	—20**	—29	90	0	0	220	—50	—18
No N	90			40			40			90			230		
N	130	40	44	60	20**	50	70	30**	75	90	0	0	270	40	17
Sig. of Int.	N.S.			N.S.			N.S.			N.S.			N.S.		
C. of V.	25.1%			14.6%			14.6%			26.0%			22.9%		
Clovers															
No G.A.	100			30			90			290			270		
G.A.	120	20	20	30	0	0	50	—40*	—44	170	—120*	—41	220	50	—18
No N	120			30			60			250			230		
N	110	—10	—8	30	0	0	70	10	17	210	—40	16	270	40	17
Sig. of Int.	N.S.			N.S.			N.S.			N.S.			N.S.		
C. of V.	27.8%			45.2%			30.1%			27.2%			22.9%		

Yields

The results given in Table 2 show a significant yield response from gibberellic acid in the first cut and a similar response from nitrogen, but no interaction when the two were applied in combination.

For the remaining four cuts gibberellic acid depressed yields compared with untreated plots; this was highly significant in two of the cuts. Nitrogen gave no response during the winter period, but a significant response in early spring which fell off again in subsequent cuts.

Botanical Composition

Herbage dissection. The results in Table 3 show the responses and depressions in yield of the main species present. These follow a similar pattern to total yields on gibberellic acid treatments.

The relative contribution of timothy, cocksfoot, and clovers to total yields was greater at the first cut on gibberellic acid treatments. The depressions recorded in these species at subsequent cuts in general exceeded that of ryegrass.

Significant nitrogen responses were obtained from the grasses up to the penultimate cut while the clovers showed a temporary although non-significant depression in yields.

Point analysis. Insufficient points were recorded for statistical analysis of the data obtained, but results are of value in showing trends in pasture composition. These results are given in Table 4.

TABLE 4. Point-analysis Results (60 points per plot)

	22.5.58				22.7.58			
	Control		Gibberellic Acid		Control		Gibberellic Acid	
	Mean Hits	% Sward	Mean Hits	% Sward	Mean Hits	% Sward	Mean Hits	% Sward
Ryegrass	217	71	284	71	87	61	81	64
Cocksfoot	9	3	14	4	3	2	2	2
Timothy	13	4	17	4	8	5	6	4
Red clover	5	2	3	1	6	4	4	3
White clover	56	18	66	17	14	9	19	15
Other grasses	7	2	13	3	27	19	15	12
Bare ground	—		—		4		8	
Total	307	100	397	100	149	100	135	100

At the first recording the percentage sward showed no variation resulting from treatment with the acid. The total hits indicate a

tendency towards increased density of ryegrass and to a lesser extent of cocksfoot, timothy, and white clover on gibberellic acid plots.

At the second recording, gibberellic acid plots showed a slight increase in the percentage of white clover and a corresponding decrease in weed grasses (mainly *Poa annua*). The individual hits also indicated this trend, but to a lesser extent.

Total-nitrogen Analyses

Results of total-nitrogen determinations on samples collected (1) prior to the first mowing, (2) at the first cut and (3) at the second cut are shown in Table 5.

TABLE 5. Total-nitrogen Levels

	% N (oven-dry material)			lb per acre N		
	30.4.58	28.5.58	31.7.58	30.4.58	28.5.58	31.7.58
Untreated	3.77	3.93	3.57		37.7	20.7
Gibberellic acid	3.90	3.89	3.94	Yield data not taken	43.2	15.8
Nitrogen	3.96	4.21	3.79		46.7	19.7
G.A. + N	4.30	3.98	3.72		47.2	20.5

No statistical analysis available.

In terms of percentage nitrogen, in the first sample a slight rise followed treatment with gibberellic acid, and a still greater rise on nitrogen-treated plots. In combination the two appear to have acted independently in raising the nitrogen level to the sum of their separate effects.

At the second sampling, gibberellic acid caused a slight lowering of the percentage nitrogen present in the herbage, while fertiliser nitrogen gave a further increase in the percentage nitrogen. In combination, the depressing effect of gibberellic acid caused a net decrease in the percentage nitrogen. In terms of nitrogen produced per acre, however, the yield response from gibberellic acid accounted for the higher yield of nitrogen per acre, and this was also reflected when applied in combination with fertiliser nitrogen.

The third sampling showed a general fall in nitrogen levels as would be expected in July, with a slightly higher percentage of nitrogen on nitrogen-treated plots compared with untreated plots. Gibberellic acid gave a surprisingly high figure for per cent nitrogen, but in terms of nitrogen produced per acre this treatment yielded less than untreated plots. On nitrogen-treated plots the yield of nitrogen per acre differed little from untreated plots.

Discussion

The results obtained in this investigation were not as precise as desirable, as shown by the rather high coefficient of variation obtained in

some of the data. Nevertheless, yield results broadly verify the work of Morgan and Mees in that marked and rapid responses were obtained from gibberellic acid. The typical colour changes and stem elongation noted by these workers were also present. These results also confirm the equal but slower-acting response from fertiliser nitrogen by the time the first cut was taken.

The depression effects of gibberellic acid both at the second cut and subsequent cuts were greater than the increase in yield obtained from this growth stimulant in the first cut. Such a result does not agree with the findings of Morgan and Mees who recorded the size of yield depressions at the second cut of the same order as response at the first cut. Further they found no significant yield difference at the third cut whereas this experiment gave a highly significant depression at the third cut. The explanation probably lies in the incapability of herbage treated with gibberellic acid to absorb available nutrients owing to the greater proportion of top growth being removed on treated plots and thereby retarding root growth (Crider 1955), as suggested by Morgan and Mees. The percentage and yield of clovers was not reduced at the second cut probably as a result of gibberellic acid increasing the length of stem and thereby allowing a greater removal of clover leaves by the mower. The stem-elongation effects disappeared by the time of the third cut and subsequent cuts when depressions in the percentage clover were evident. This is confirmed by point analysis on 22 July where the number of hits on clovers was not greatly affected by gibberellic acid treatment.

There is no statistical evidence to suggest an interaction between gibberellic acid and nitrogen. The yield figures shown in Table 2 for individual treatments strongly suggest an independent effect of gibberellic acid when applied in combination with fertiliser nitrogen.

The nitrogen levels in the herbage following gibberellic acid treatment follow the general pattern of crude protein levels found by Morgan and Mees.

Conclusion

This investigation has shown a similarity of results obtained from the use of gibberellic acid under New Zealand conditions with those obtained in England.

The more rapid growth responses obtained from gibberellic acid compared with fertiliser nitrogen applied in autumn would be of little practical use because of the subsequent depressions in growth from gibberellic acid in the winter, while nitrogen fertiliser at this time is giving growth responses. Applied in spring, gibberellic acid could be of value for providing a bulk of feed quickly, but the practicability of such usage in New Zealand's agriculture will have to await a substantial lowering in cost of gibberellic acid.

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FATE OF POTASSIUM APPLIED TO PASTURE ON A SOIL DERIVED FROM ANDESITIC ASH

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Summary

A pot experiment was used to study the problem of short-lived responses to potassium on yellow-brown loam soils of North Taranaki, derived from fine-textured andesitic volcanic ash. Undisturbed cores of soil carrying original pasture were treated with sheep or cow urine at rates corresponding to one or two urinations, the potassium added in the urine being approximately equivalent to 8 and 16 cwt/ac of KCl. KCl was applied at 2, 4, and 8 cwt/ac. Results, over a period of six months from spring to autumn, were as follows.

The K content of both grass and clover was increased by the treatments, approximately in proportion to the amount added, with both KCl and urine. A large growth response to the urine applications was obtained, attributable mainly to nitrogen. A small growth response to KCl was obtained; this was the same at all rates of KCl and was entirely due to increased growth of clovers. Topdressing with KCl greatly increased the percentage of clovers in the pasture. Because of increased growth and K content, a large proportion of the added K was taken up by the pasture in all treatments.

Losses of K by leaching were comparatively small, and practically all occurred within three months. Appreciable amounts of Ca and Mg were found in the leachates from all treatments. The amounts of Mg lost indicated that continued topdressing with potassium could possibly result in a deficiency of magnesium in some soils. The level of exchangeable K in the soil at the beginning of the experiment was slightly above the accepted response level of 0.55 me.%. After six months the control and the 2 and 4 cwt/ac KCl treatments showed deficient levels of exch. K, the single urination treatment was on the borderline, and only the 8 cwt/ac KCl and double urination treatments were still above the response level. At the normal rate of topdressing of 2 cwt/ac of KCl, uptake of available soil and fertiliser K by the pasture was so rapid that a deficiency level of K in the pasture was reached within three months. The effects of a single urination on soil and plant K had completely disappeared at the end of six months. It is concluded that the short effective period of water-soluble potassium fertilisers on soils of the yellow-brown loam group under pasture is due principally to luxury uptake of applied potassium by the pasture, and its concentration by the grazing animal within the small areas covered by urine patches.

Introduction

Much of the intensively grazed pasture land used for dairying and fat lamb production in the North Island of New Zealand is located on soils derived from fine-textured andesitic and rhyolitic volcanic ash, and classified as yellow-brown loams (Taylor 1948; N.Z. Soil Bureau 1954; Taylor and Cox 1956). The yellow-brown loams, generally speaking, are markedly potash-responsive, and the annual application of 1 to 2 cwt of muriate of potash is becoming more or less standard practice (Burgess and Davies 1951; Burgess 1958). Responses to potash under

grazing conditions, however, are generally short-lived (A. C. Burgess, pers. comm.).

In the yellow-brown loams, and in most other soils formed from volcanic parent materials in New Zealand, 2 : 1-type (micaceous) clay minerals are virtually absent and the clays are generally of the allophane type with smaller amounts of 1 : 1-type minerals of the kaolinite-halloysite group (Fieldes and Swindale 1954). With these latter minerals it is unlikely that any mechanism exists for holding potassium in a fixed (i.e., non-exchangeable but potentially available) form which might act as a buffer system in the soil with respect to potassium release and fixation. In these soils, therefore, replenishment of exchangeable potassium depends mainly on the weathering of primary minerals such as volcanic glasses and feldspars. It is apparent, however, from the responses to potassium generally observed on these soils, that the rate of release of potassium by weathering of primary minerals is quite inadequate to replace that lost from the exchangeable form. The lack of K-fixing minerals, allied to high rainfall and free drainage, in the dairy pastures of the Taranaki and Waikato districts, where yellow-brown loams predominate, would further be expected to result in a significant loss of potassium by leaching. Two more generally applicable factors, which also may be expected to aggravate losses of potassium are, firstly, luxury uptake by the pasture, and secondly, the uneven return of potassium to the soil in the urine of the grazing animal. If these suppositions are correct it is hardly surprising that responses to potassium on soils such as the yellow-brown loams are apparently so fleeting.

In the work described in the present paper an attempt has been made to evaluate more precisely, by means of a pot experiment, the fate of potassium added as animal urine or as potassium chloride to a yellow-brown loam under a grass/clover pasture.

Experimental

The soil used was the New Plymouth black loam from an experimental area at Bell Block. Some general chemical characteristics for 0–6 in. depth are: pH, 5.9; c.e.c., 39.5 me.%; % b.s., 67%; exch. Ca. 22 me.%; exch. Mg, 3.1 me.%. Undisturbed cores to a depth of 6 in., carrying a mixed pasture, were placed in dark glass jars 11.8 cm in diameter, each being placed over a glass dish which collected the leachate. Evaporation and contamination of the leachate were minimised by enclosing the dish and lower part of the jar in a polythene bag. Before treatment, the pots were left for about a month to settle down, and they were then rated in four groups according to the quality of the pasture. One pot from each group was selected to make up the four replicates used for each treatment. No basal dressing was applied because the soil had already been topdressed with superphosphate.

Treatments

(1) *Control* (treatment C)—100 ml of water.

(2) *Sheep's urine, single dose (US1)*—70 ml of sheep urine containing 483 mg K.

(3) *Cow's urine, single dose (UC1)*—130 ml of cow urine, containing 709 mg K.

(4) *Sheep's urine, double dose (US2)*—Two 70-ml portions of sheep's urine, the first added at the commencement of the experiment and the second 28 days later, containing in all 966 mg K.

(5) *Cow's urine, double dose (UC2)*—Two 130-ml portions of cow's urine, the first added at the commencement of the experiment and the second 28 days later, containing in all 1418 mg K.

(6) *Muriate of potash, at 2 cwt/ac (K2)*—144 mg of K, added as KCl, in 100 ml of water (= 117 lb/ac of K; or 2 cwt/ac muriate of potash (60% K_2O)).

(7) *Muriate of potash, at 4 cwt/ac (K4)*—288 mg of K, added as KCl, in 100 ml of water (= 234 lb/ac of K; or 4 cwt/ac muriate of potash).

(8) *Muriate of potash at 8 cwt/ac (K8)*—576 mg K, added as KCl in 100 ml of water (= 467 lb/ac of K; or 8 cwt/ac muriate of potash).

No nitrogen was added with the KCl because these treatments were intended to represent topdressing with potassium fertiliser when normally no nitrogen is added.

It was found that the only differences between the effects of sheep and cow urine were ones of degree, so to simplify presentation of the results, data for treatments 3 and 4 (US1 and UC1), and 5 and 6 (US2 and UC2), have been combined, and the urine treatments are henceforth referred to as U8 (single application) and U16 (double application), receiving respectively 596 and 1192 mg K per pot. The potassium equivalent of these is approximately 483 and 966 lb/ac of K, or 8.6 and 17 cwt/ac of muriate of potash.

The pots were set up in a wire-netting enclosure with a Perspex roof, treatments were applied on 29 October 1957, and growth of pasture continued for six months. Leaching of the pots was carried out by adding 100 ml of water on each of six days of each week. This gave an effective leachate for the control of about 15 in. of water (Table 4) in the six months of the experiment, which is approximately half of the annual surplus-water figures of 33.7 in. for New Plymouth, calculated according to Thornthwaite's rational index (Hurst 1951).

Analyses

At intervals of 28 days the leachates were measured and analysed for K, Ca, and Mg. At the same time the pasture was clipped short, separated into grasses and clovers, oven-dried (105°c), and weighed. No clippings were returned to the pots. Yields from replicate pots were recorded separately, but replicates were bulked for chemical analysis. The oven-dried material, after wet ashing with nitric and perchloric acids, was analysed for K, Ca, Mg, and P.

After six months the soil cores were removed from the pots and cut into layers of 0–2, 2–4, and 4–6 in. Replicates were bulked, air-dried and weighed, and retained for chemical analysis. Moisture content on the air-dry sample was determined by oven-drying at 105°c. Exchangeable cations were determined in ammonium-acetate leachates according to Metson (1956).

Results

General Observations

Effect of Urine. A response to urine was apparent within fourteen days, the pasture being dark green and very dense and vigorous compared with that in the control and KCl pots. Growth was much greater, reaching a maximum for the single application of urine (U8) two months after treatment (Fig. 1a). At the end of the fourth month the pasture on these pots was becoming yellowish and in the fifth month was a similar yellowish green to the controls. The second application of urine 28 days after the first (U16) killed the leaves of the pasture, and 14 days later only a few tillers were growing, but these were very vigorous. This sparse but vigorous growth showed a small response in production of dry matter at the end of the second month. The response for this treatment reached a maximum three months after addition of the second increment, and thereafter declined (Fig. 1a). In the fifth month the pasture was still green, but in the sixth it was becoming yellowish.

Effect of KCl. The response to KCl at all levels was small compared with that to urine, and reached a maximum in the fourth month. The response was due almost entirely to the clover (Fig. 1c); over the entire period the grass showed no response (Fig. 1b). The higher rates of KCl (K4 and K8) gave no greater production of dry matter than did the 2 cwt application (K2).

Clover response. KCl topdressing greatly increased the proportion of clover in the pasture (Table 1). Three weeks after treatment the pasture appeared clover-dominant, but at this stage the dry-matter weights showed no increase in growth or in the proportion of clover in the pasture. By the end of the second month there was a small growth response and the proportion of clover had increased. A maximum was reached in the KCl treatments at about four months, the values being 45%, 62%, and 57% of clover for the K2, K4, and K8 treatments respectively (Table 1). In the K2 treatment the proportion of clover had fallen to 22% (the overall average for the control) by the sixth month.

In the urine treatments any effect of potassium in stimulating clover growth was more than matched by the stimulation of grass growth by the nitrogen, although the total yield of clover was higher than in the control, particularly in U16 (Table 1).

Uptake of Plant-nutrient Elements by Pasture

Potassium. All treatments considerably increased the potassium content of both grass and clover (Figs. 2b, 2c; Table 2). The K content of the control pasture was very low (1.19% K) at the beginning

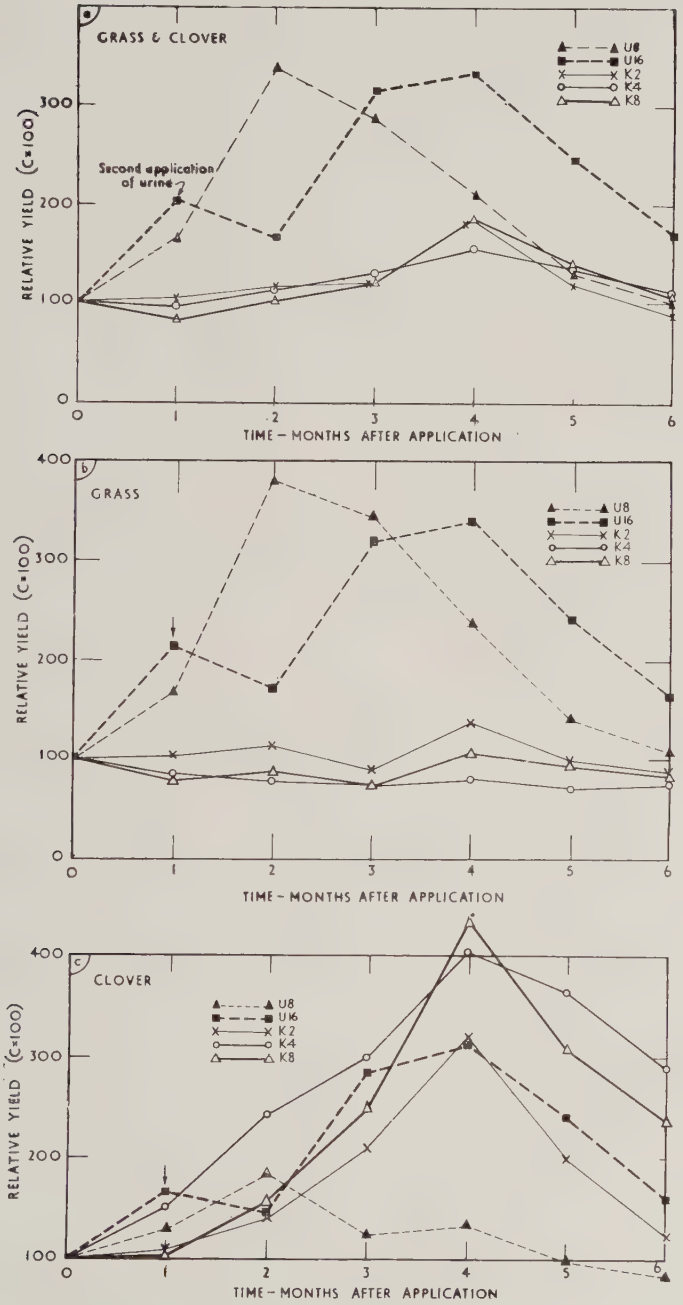

Fig. 1.—Growth response of pasture to applications of urine or KCl.

TABLE 1. TOTAL YIELD OF GRASS AND CLOVER AND PERCENTAGE OF CLOVER

	Treatment							
	C	U8	U16	K2	K4	K8	¾ C + ¼ U8 (calcd)	¾ K2 + ¼ U8 (calcd)
Total yield of grass (g/pot)*	4.88	10.66	11.94	5.10	3.73	4.20	6.32	6.49
Total yield of clover (g/pot)	1.34	1.70	3.12	2.62	4.04	3.51	1.44	2.39
Grass and clover (g/pot)	6.22	12.36	15.06	7.72	7.77	7.71	7.76	8.88
Grass and clover (lb/ac)	5,040	10,020	12,200	6,250	6,290	6,250	6,290	7,190
Percentage of yield† as clover:								
1st month (Nov.)	17	13	14	18	27	22	16	17
2nd month (Dec.)	23	13	20	27	48	35	21	24
3rd month (Jan.)	26	12	25	45	60	55	23	36
4th month (Feb.)	24	16	23	43	62	57	22	37
5th month (Mar.)	23	16	18	37	60	49	20	31
6th month (Apr.)	16	14	16	22	42	36	16	20
Percentage of total yield† as clover	22	13	21	34	52	46	20	29

* Oven-dry (105°c) weights.

† Based on oven-dry weights.

of the experiment (Fig. 2a) and well below the critical level of about 2.3% K proposed by McNaught (1958) for good growth of a mixed grass-clover pasture. Averaged over the period of the experiment, the K contents of grass and clover in the control were only 0.97% and 0.63% K.

TABLE 2. CHEMICAL COMPOSITION OF GRASS AND CLOVER
(calculated on total production over six months)

	Treatment							
	C	U8	U16	K2	K4	K8	¾ C + ¼ U8 (calcd)	¾ K2 + ¼ U8 (calcd)
Potassium (%K*)								
in grass	0.97	2.98	3.58	1.95	2.29	2.88	1.48	2.21
in clover	0.63	2.94	3.73	1.72	2.79	3.46	1.21	2.03
in grass and clover	0.90	2.97	3.61	1.87	2.55	3.14	1.42	2.08
Calcium (%Ca*)								
in grass	0.94	0.59	0.57	0.79	0.91	0.74		
in clover	1.58	1.22	1.25	1.45	1.52	1.24		
in grass and clover	1.08	0.68	0.71	1.02	1.23	0.97		
Magnesium (%Mg*)								
in grass	0.51	0.36	0.38	0.50	0.49	0.45		
in clover	0.68	0.46	0.48	0.61	0.53	0.53		
in grass and clover	0.55	0.37	0.40	0.54	0.51	0.49		
Phosphorus (%P*)								
in grass	0.36	0.25	0.22	0.40	0.45	0.37		
in clover	0.34	0.24	0.24	0.30	0.32	0.30		
in grass and clover	0.36	0.25	0.22	0.37	0.38	0.34		

* Based on oven-dry (105°c) weights)

In treatment U8 (single application of urine) the K content of both grass and clover reached a maximum in the second month with values of 3.85% and 3.35% K respectively, and the subsequent decline in K content was more pronounced in the grass than in the clover (Figs. 2b, 2c). In U16 the maximum K content was reached in the second month (i.e., a month after the second application of urine) for the clover and in the third month for the grass, and subsequently remained at a higher level than in U8.

In treatments K4 and K8, the K content of the clover was higher than that of the grass throughout the experiment. In K8 the K content of both clover and grass was well maintained right up to the sixth month at about 3% (Figs. 2b, 2c). With the 4 cwt/ac application (K4) the K content of the clover was also well maintained, falling

only to 2.55% at six months, with the K content of the whole pasture at 2.35% (Fig. 2a) —just approaching McNaught's (1958) critical value. Thus, the higher KCl applications (K4 and K8) compared well with the urine treatments as regards concentration of K in the herbage, but the total uptake of K (Table 3) was much less because of lower production of dry matter.

TABLE 3. TOTAL UPTAKE OF K BY GRASS AND CLOVER

	Treatment					
	C	U8	U16	K2	K4	K8
Potassium in grass (mgK/pot)	47.3	318	427	99.5	85.4	121
in clover (mgK/pot)	8.5	49.9	116	45.1	113	121
in grass and clover (mgK/pot)	55.8	368	543	145	198	242
Total K added (mg/pot)	—	596	1192	144	288	576
Percentage of added K recovered in pasture	—	52	41	62	49	32

With the lowest rate of KCl—2 cwt/ac KCl, treatment K2—which corresponds more nearly to farm practice, the K content of the grass reached a maximum (2.6%) in the second month (Fig. 2b). The clover reached a maximum K level (2.85%) in the first month, which was nearly maintained (at 2.7%) for the second month (Fig. 2c). Thereafter, the K level in both grass and clover, particularly the latter, declined rapidly, reaching the very deficient level of 1.35% after six months, with the K level in the clover consistently lower than in the grass. It would appear from these results that, given adequate potassium, the clover was able to mantain at least as high a level of K in the leaves as the grass. When potassium was inadequate, however, the K content of the clover fell below that of the grass.

The decrease in K content of the clover in K2 in the third month (Fig. 2c) coincided with the maximum percentage of clover in the pasture (Table 1), which was also the stage at which the K content of the whole pasture (Fig. 2a) began to fall below the critical value of 2.3% K given by McNaught (1958). The maximum dry-matter production, however, was not reached until the fourth month (Fig. 1a), when the K content of the whole pasture had fallen to the deficient level of 1.65%.

The total uptake of potassium was considerably increased by all treatments (Table 3), owing to increased growth and higher K content

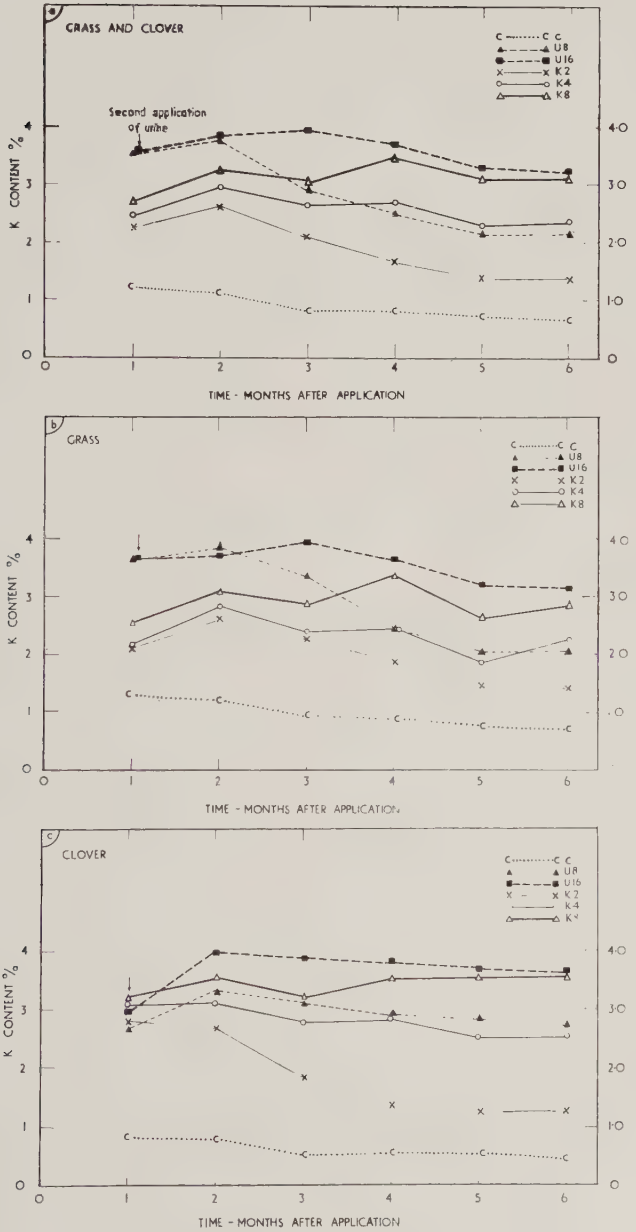

Fig. 2.—Potassium content of pasture after application of urine or KCl.

of the pasture. The recovery of potassium by the pasture accounted for a large proportion of that added (up to 62% for K2).

Calcium and Magnesium. The urine treatments lowered the Ca and Mg concentration in the pasture (Table 2), but these values were well above deficiency levels. Little change was observed over the period of the experiment. The KCl treatments lowered the values slightly; no consistent effect on Ca content was observed with increments of KCl added, but Mg content tended to decrease from K2 to K8.

Phosphorus. The phosphorus level was lowered by the urine treatments, an effect probably due to nitrogen (Table 2). Values for the whole pasture decreased to a minimum of about 0.19% P three months after urine was applied, and then increased again toward that of the control. This fluctuation may have followed a build-up of nitrate N by nitrification of urea, and subsequent loss of nitrate by leaching and uptake by the pasture. The KCl treatments had little effect on the phosphorus content of grass or clover (Table 2).

TABLE 4. Analysis of Leachates

	Treatment							
	C	U8	U16	K2	K4	K8	¾ C + ¼ U8 (calcd)	¾ K2 + ¼ U8 (calcd)
Volume of leachate (litres/pot)	4.4	2.5	2.5	3.9	4.3	4.1		
Volume of leachate (in.)	15.7	9.1	8.9	13.8	15.3	14.5		
Total K added (mg/pot)	—	596	1192	144	288	576	149	257
Total K in leachate (mg/pot)	1	41	164	3	12	81	11	12
Percentage of added K in leachate	—	8	15	1	4	14	7	4
Total Ca in leachate (mg/pot)	7	85	468	24	64	103	23	8
Total Mg in leachate (mg/pot)	2	20	53	6	15	27	6	9
K in leachate lb/ac	1	33	133	2	10	66	9	10
Ca in leachate lb/ac	6	69	379	19	52	83	22	32
Mg in leachate lb/ac	2	16	43	5	12	22	5	8

Losses of Potassium, Calcium, and Magnesium in the Leachates

Potassium. Losses of potassium (Fig. 3a; Table 4), as would be expected, increased with increasing amounts of K added. Except for treatments U16 and K8, which are outside normal farming experience, losses of K by leaching were not particularly high and in all treatments were negligible after the third month. At the 2 cwt/ac rate (K2), which is a moderately heavy application on pasture by New Zealand standards, the loss of K was barely measurable and even in treatment K4 it amounted to only 4% of that added, or some 9 lb/ac of K. In treatment K8 the loss amounted to 66 lb/ac, but in U8 receiving a similar application of K it was only 33 lb, because of greater pasture growth in the latter.

It should be noted that the results for the U8 and U16 treatments are not applicable to the whole area of a pasture, but only to places receiving respectively a single or a double urination, which may amount at the most to some 25% of the total area in the course of a year. On the other hand, although the equivalent of six months' moisture surplus was put through the pots, this was done during the main part of the growing season and it is probable that greater losses of K than those indicated would be experienced during the winter from fresh urine patches.

Calcium and Magnesium. Quite appreciable amounts of calcium and magnesium were found in the leachates from the urine and KCl treatments (Figs. 3b, 3c; Table 4). These fell away with time in the same way as the potassium in the leachates; moreover, in the series K2, K4, K8, the losses of Ca and Mg were almost directly proportional to the amount of K added.

Exchangeable Potassium

Exch. K values for the 0–2, 2–4, and 4–6 in. depths at the beginning and at the end of the experiment are given in Table 5. Included also are estimates of the values for the 0–6 in. and 0–3 in. depths (the latter for comparison with Hogg's (1957) results which were on this basis). If Hogg's response figure of 0.55 me.% K, for the 0–3 in. layer of North Island pasture soils, is taken as a reference level, the initial value for the soil used in this experiment was slightly above this, at 0.67 me.%. By the end of the experiment—that is, within six months—this had fallen to the very low figure of 0.28 me.% for the control, while treatments K2 and K4 were also in the K-deficient range. Treatment U8 was on the borderline at 0.55 me.%, while only U16 and K8 (treatments which would seldom be met with in practice) were still above it. Treatment U8 showed a much lower level of exch. K at all depths than did K8 in spite of the fact that more K was lost by leaching in the latter (Table 4); obviously the greater growth in U8 caused a bigger drain on the exch. K in this treatment. Other points which emerge from the figures are the rapidity with which the exch. K value changes in this soil, an effect shown even in the control, and the striking way in which, in all treatments, the exch. K value diminished with depth.

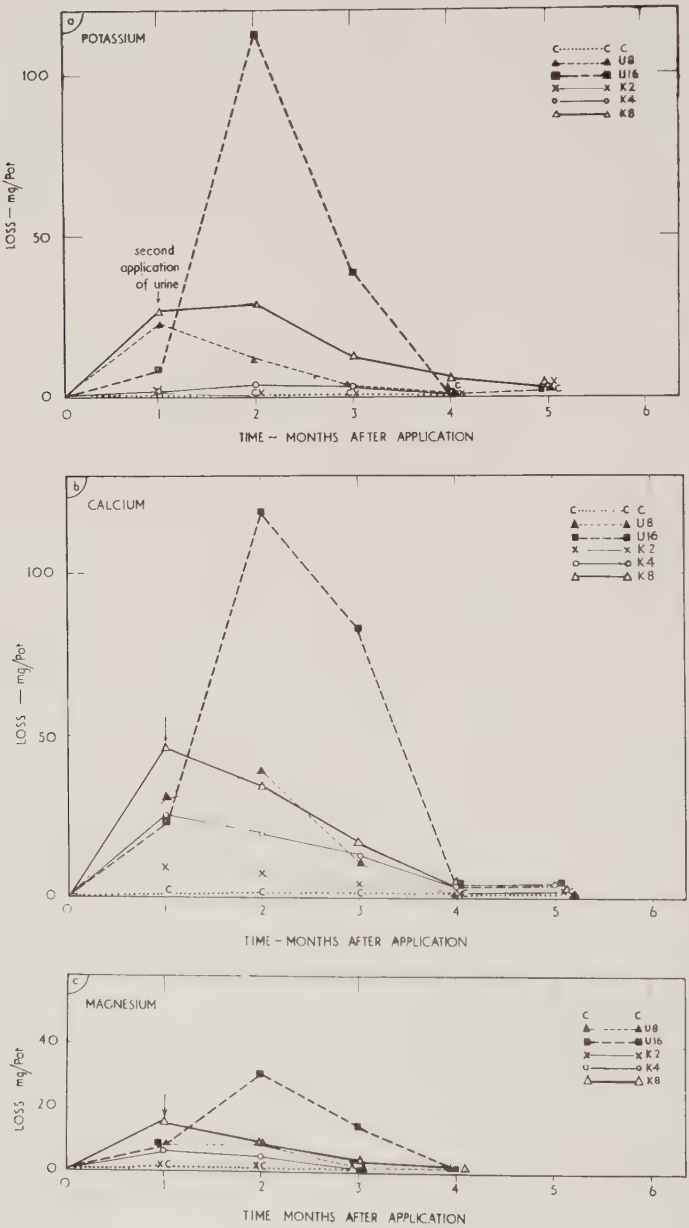

Fig. 3.—Potassium, calcium and magnesium in leachates after application of urine or KCl.

TABLE 5. EXCHANGEABLE POTASSIUM CONTENT OF SOILS

	Depth (in.)	Before Exp.	Treatment							
			C	U8	U16	K2	K4	K8	¾ C + ¼ U8 (calcd)	¾ K2 + ¼ U8 (calcd)
Exch. K (me.%)	0–2	0.82	0.33	0.63	1.06	0.40	0.43	0.88	0.40	0.46
Exch. K (me.%)	2–4	0.37	0.19	0.40	0.71	0.24	0.27	0.63	0.25	0.28
Exch. K (me.%)	4–6	0.19	0.19	0.22	0.37	0.14	0.23	0.45	0.13	0.16
Exch. K (me.%, estimated)	0–3	0.67	0.28	0.55	0.94	0.35	0.38	0.80	0.35	0.41
Exch. K (me.%, estimated)	0–6	0.47	0.21	0.42	0.71	0.26	0.31	0.65	0.26	0.30
Exch. K (mg/pot)	0–6	139	64	125	225	79	106	216	79	91
Change in exch. K (mg/pot)	0–6		−75	−14	+ 86	−60	−33	+ 77	−60	−48
Net change* in exch. K (mg/pot)	0–6			+61	+161	+15	+42	+152	+15	+27
Total K added (mg/pot)			—	596	1192	144	288	576		
Recovery of added K as exch. K (%)			—	10	14	10	15	26		
Change in exch. K (lb/ac)	0–6		−61	−11	+ 70	−49	−27	+62	−49	−39

* i.e., change in exch. K for treatment minus change in exch. K for control.

In Table 5, changes in exch. K have been expressed as milligrammes per pot. Assuming that the same depletion of the initial exch. K as in the control occurred in all treatments, the net recovery of the added K in exchangeable form has been calculated and expressed as a percentage of the amount added. These recoveries are comparatively small. Proportionately a greater retention of added K in the exchangeable form was shown in the KCl treatments, which was evidently due, as previously mentioned, to the lesser growth and uptake of K in these.

TABLE 6. Recovery of Added Potassium

	Treatment							
	C	U8	U16	K2	K4	K8	¾ C + ¼ U8 (calcd)	¾ K2 + ¼ U8 (calcd)
Total K added (mg/pot)		596	1192	144	288	576	149	257
K in leachate (mg/pot)		40	163	2	11	80		
K in pasture (mg/pot)		312	488	89	142	187		
K in soil, exch. (mg/pot)		61	161	15	42	152		
Total K recovered (mg/pot)		413	812	106	195	419		
Percentage of added K recovered		69	68	73	68	73		
K removed in leachate and pasture (mg/pot)		352	651	91	153	267	88	163
K removed in leachate and pasture (lb/ac)		285	528	74	124	216	71	132

Total Recovery of Added Potassium

In Table 6, recoveries of added potassium are summarised. It will be seen that potassium recovered in the leachates, in the pasture and in exchangeable form in the soil, adds up to only about 70% of that added. Because of the nature of the soil colloid it was not expected that any fixation of potassium would have occurred, but this point was checked by making extractions of various samples with boiling 0.2 N and 1 N nitric acid. Although some additional potassium was thereby extracted, this was the same for the control as for the treated soils, so that no evidence was obtained of any fixation of the added potassium in non-exchangeable form. Other possible sources of error, such as sorption of K by polythene and secretion of sap from the pasture

between clipping and oven-drying, were also explored without success. However, it is felt that these discrepancies do not invalidate the general conclusions to be drawn from the experiment.

DISCUSSION

Responses to Potassium under Field Conditions

The experiment described, being conducted under highly artificial conditions, obviously cannot be directly related to farming problems without some reservations. For example, it is impossible to scale a farm paddock down to an experimental pot, firstly because the effect of the grazing animal cannot be adequately simulated, and secondly because soil in a pot is not subjected to the same climatic and drainage conditions as in the field. These may seem obvious points, but it is as well to stress them before discussing the implications of the results. In the field, from the viewpoint of the utilisation of potassium, part of the area corresponds to either the control treatment (i.e., no K added) or to treatment K2, for example. The remainder comprises urine patches corresponding to treatment U8. Figures from the control and K2 treatments have been combined with those from U8, therefore, in an attempt to get a more realistic estimate of the trends in a grazed pasture. This is, admittedly, a somewhat crude expedient, but it does allow of a rough extrapolation from the experimental pot to the field. The percentage of the total area (25%) affected by urination in the course of a year has been derived from data of Hancock (1950) and Doak (1952), assuming 0.75 cows per acre, 9 urinations per day on the pasture, an area of 650 sq. in. for each urination, and no coincidence of urine patches.

Now, taking the hypothetical treatments ($\frac{3}{4}$ C + $\frac{1}{4}$ U8) and ($\frac{3}{4}$ K2 + $\frac{1}{4}$ U8) to represent the situations where no KCl and 2 cwt/ac of KCl respectively* have been added to a grazed pasture, the results suggest that in the absence of added fertiliser K, the effect of urine has been to produce an overall increase in dry-matter production of about 25% over that of a similar non-fertiliser area receiving no urine (Table 1, treatments C and $\frac{3}{4}$ C + $\frac{1}{4}$ U8). This increase is attributable mainly to the nitrogen of the urine, and little change in clover content has occurred. With added potassic fertiliser, however (treatment $\frac{3}{4}$ K2 + $\frac{1}{4}$ U8), the corresponding increase over the control area (i.e., no urine and no fertiliser K—treatment C) has been increased to 43% (Table 1, treatments C and $\frac{3}{4}$ K2 + $\frac{1}{4}$ U8), while the clover percentage also shows an increase. The net effect of the application of 2 cwt/ac of KCl then, under grazing conditions, has been to give a relative increase in dry-matter production of [(143–125)/125] × 100, or 14%, due mainly to stimulation of clover growth (Table 1), compared with a similar area receiving no fertiliser K.

* The latter situation would more accurately be represented by (K2 + $\frac{1}{4}$ U8), from the point of view of the amount of K added, but it is felt that this would give an overestimate of the effectiveness of a K2 application on a grazed pasture (since fertiliser K would be without effect on urine patches because of the large amount of K already present); the approximation ($\frac{3}{4}$ K2 + $\frac{1}{4}$ U8) has therefore been preferred.

The use of higher rates of potassium (corresponding to K4 or K8) in order to maintain an adequate K status of the soil (Table 5) and pasture (Fig. 2a) for a longer period would seem to be of doubtful merit. In the present experiment, dry-matter production declined at the same time in treatments K4 and K8 as in K2, and total production was no higher (Table 1). Moreover, over the period of the experiment, losses of K by leaching and uptake were greater from K8 and K4 than from K2—216 and 124 lb/ac as compared with 74 (Table 6)—militating against a proportionately longer period of effectiveness for the higher rates of application.

Clover Growth

The effects of added potassium on the clover component of a mixed pasture in the present experiment are in general agreement with what has been found by other workers. In New Zealand, McNaught (1954, 1958) has found that, where availability of potassium is a limiting factor, potash deficiency is shown first by the clovers. Lack of vigour in the clovers in turn has repercussions on the nitrogen supply of the associated grasses. In America, Blaser and Brady (1950) have shown that maintenance of the legume in a grass/legume association may be very difficult to achieve where the potassium status of the soil is low. McNaught (1958) in plot experiments on Horotiu sandy loam (a yellow-brown loam on rhyolite alluvium and andesitic ash) found the critical level (minimum level for near-maximum growth of white and red clover for whole leaves) to be 1.8% K, while the value for ryegrass was 1.6% K. However, because of the competition of the grasses, "if clovers in a mixed pasture are to take up their minimum requirement of 1.8% K for optimum potassium nutrition, the associated ryegrass or other high-producing grass can be expected to contain about 2.3% K" (McNaught, *loc. cit.*).

In the present work, apart from K values for the control which were invariably deficient for both grass and clover according to McNaught's criteria, critical levels were reached only by the clover of the K2 treatment (from the fourth month onwards), and by the grass of the same treatment, which was just below McNaught's 1.6% critical level for ryegrass in the fifth and sixth months (Figs. 2b, 2c). Thus, although the total dry-matter production was the same throughout the series K2, K4, K8, it may well be indicative of an incipient (or actual) potassium deficiency in K2 that the clover percentage dropped much more sharply and to a much lower figure in this treatment than in K4 and K8.

The Grazing Animal

The grazing animal adds to the problem of utilisation of potassium by collecting K from the whole area of the paddock and concentrating the greater part of this ingested K within the relatively small area of the urine patches. Concentration of fertiliser K in this manner is assisted by rapid "luxury" uptake by the pasture. The urine patches, in addition, receive an equally extravagant application of nitrogen which encourages vigorous growth of grass and by comparison restricts clover growth and nitrogen fixation. The net result, in potassium

responsive soils, is that the areas not receiving urine tend to become potassium deficient again within three to four months of the application of the fertiliser. A nitrogen deficiency is also likely to result because of a decline in the proportion and vigour of the clover in the pasture.

The rate of loss of potassium by leaching is undoubtedly increased by concentration in the "urine patches", but it must be remembered that these areas comprise only a fraction—perhaps one-quarter—of the whole area of pasture in any one year. Estimates of the overall loss (Table 5) amounted to about 10 lb/ac of K for ($\frac{3}{4}$ C + $\frac{1}{4}$ U8) and ($\frac{3}{4}$ K2 + $\frac{1}{4}$ U8), for the six months of the experiment (at the end of which leaching losses in all treatments had become negligible). This figure would probably need to be increased somewhat to allow for greater losses from urine patches formed during the winter months, when there is little growth to utilise the soluble K and when leaching by percolating rainwater is likely to be at a maximum (see, for example, the loss of K after the second application of urine in U16—Fig. 3—when growth was temporarily suspended). For this reason, application of potassic fertilisers in the late autumn (April and May) would seem to be inadvisable.

The exch. K level in the soil with the single urine treatment (U8), six months after application, was below the level in the soil prior to the experiment, and had, in fact, just reached Hogg's (1957) response level of 0.55 me.% (Table 5). Hence, until another application of urine is received, or fertiliser K is added, such an area would very soon become potassium-deficient. Under normal grazing conditions a fresh increment of urine would not be expected, on the average, until about four years had elapsed. The inescapable conclusions are that, despite the large amount of potassium received, the urine patch on this particular soil does not provide a long-term reserve of potassium, and furthermore that, at a given time, not more than about 15% of the grazing area would consist of urine patches of adequate potassium status.

Finally, applying this reasoning to the design of field experiments, it can be seen that the common practice of returning pasture clippings uniformly over the experimental area to simulate the return of plant nutrients by the grazing animal is fundamentally unsound. It would appear preferable to devise a method of returning a concentrate or extract of the clippings in a non-uniform manner, as in "urine patches", or failing this, to discard them altogether, since it is the pasture between the urine patches that is of most interest in such experiments.

Role of Nitrogen

The effect of nitrogen on responses to potassium in pasture soils is a question that does not seem to have received adequate attention. Although much has been made of the high efficiency of clovers in nitrogen fixation in New Zealand grass/clover pastures, and of the massive returns of nitrogen to the sward by way of the urine and dung of the grazing animal, there has been much less attention paid to the uneven return of this nitrogen. Thus, it is quite possible that the areas between the urine patches will at times suffer from a deficiency

of nitrogen (as well as of potassium), because of lack of vigour in the clovers. In the present experiment the slightly yellowish and unthrifty appearance and comparatively low production of the KCl pots was in marked contrast to the deep green appearance, strong growth and high dry-matter production of the urine-treated pots. The question naturally arises as to whether the response of pasture growth to fertiliser K can economically be increased by adding nitrogen also. The economics of nitrogen fertilisation are generally dismissed as unfavourable for New Zealand pastures, but this opinion may need to be reconsidered in circumstances where the grasses are suffering from lack of vigour in the clovers.

Losses of Magnesium

Under normal circumstances, regular application of superphosphate and occasional liming would undoubtedly more than make good any potassium-induced leaching losses of calcium from pasture soils. With magnesium the situation is rather different, because magnesium is usually present in relatively small amount as exchangeable ion, and is not normally added as a fertiliser (except in "serpentine super" in which its immediate availability is not yet adequately known). The displacement of exchangeable Mg by either Ca (in lime and in superphosphate—Dixon and Taylor, 1942) or K (in KCl, or in animal urine, as shown in the present work) could therefore lead in time to a serious depletion of exchangeable magnesium, in some soils at least.

In the present experiment the leaching loss of Mg in treatment ($\frac{3}{4}$ K2 + $\frac{1}{4}$ U8) amounted to about 9 mg/pot (Table 4), which is equivalent to approximately 0.1 me.% Mg for the 0–6 in. layer of soil. This is not high in comparison with the total exch. Mg content of this soil of 300 mg/pot or 3.1 me.%, but it is a far from negligible proportion. At the K4 rate the corresponding loss was about 15 mg/pot, or 0.15 me.% which is quite appreciable. Indeed, in soils with a low level of exch. Mg there is every possibility that continued topdressing with potassium—which is unquestionably necessary in any case on the kind of soil under discussion—will in the comparatively near future result in a deficiency of magnesium also. Although definite experimental evidence of the limitation of pasture or lucerne growth owing to magnesium deficiency is so far lacking in New Zealand, this is nevertheless regarded as a distinct possibility. The situation may be regarded as analogous to that obtaining in apple orchards of the Nelson district, where premature defoliation of apple trees attributable to magnesium deficiency was brought about by heavy applications of potassic fertilisers on soils originally low in exchangeable magnesium (Kidson *et al.* 1940).

There may be a further danger here in that temporarily high levels of exchangeable potassium, in conjunction with low levels of exchangeable magnesium, may be conducive to the development of hypomagnesaemia (low Mg levels of the blood serum) and grass tetany. Work in Holland particularly has shown that the onset of this distubrance in milking cows is usually associated with high potassium topdressings (sometimes aggravated by high nitrogen also), particularly in the spring and autumn (Kemp and t'Hart 1957; Kemp 1958).

CONCLUSIONS

Although the present experiment was carried out on a yellow-brown loam in Taranaki, the results may reasonably be regarded as having a wider application. In the first place, from the point of view of uptake and conservation of potassium, no important distinction can be made between cattle and sheep grazing. Secondly, year-round grazing on permanent grass/clover pastures is general in New Zealand. Hence, in looking at the problem on a country-wide basis, the main variable to be considered is the nature of the soil.

On New Plymouth black loam, return of potassium in urine, at an estimated average annual rate for the whole pasture of 2 cwt/ac of KCl, is evidently far from adequate to maintain the potassium status of the soil and pasture. Lacking 2 : 1-type clay minerals this soil is not only unable to release appreciable potassium by weathering but is also unable to conserve added soluble potassium by fixation. It follows, then, that other soils lacking clay minerals of the 2 : 1 or micaceous type are likely to behave similarly. In New Zealand, these include most of the intrazonal soils on volcanic parent materials, namely: the yellow-brown loams on fine-textured andesitic and rhyolitic ash; the yellow-brown pumice soils on coarse, little-weathered rhyolitic pumiceous ash; the brown granular loams and clays on older andesitic ash and andesite; and the red and brown loams on basalt scoria and basalt flows (Taylor 1948; Fieldes and Swindale 1954; Taylor and Cox 1956). All of these soils are normally responsive to potassium and can be expected to remain so because of their low potassium-supplying power (Metson *et al.* 1956), and by their lack of K-fixing minerals. Similar considerations apply also to the yellow-brown sands, which occur mainly in western coastal areas of the North Island, and to the organic soils. In all of these soils, potassium deficiency, once apparent, may be regarded as a chronic condition which can be alleviated by periodical additions of potassium, but not cured, because there appears to be no practicable way of permanently building up the potassium status of the soil.

The situation is more complex on soils in the zonal category, which "are derived from sediments and shales which are similar in nature to acid-intermediate rocks and contain varying amounts of mixed micas, feldspars, and quartz, with small amounts of more basic minerals" (Fieldes and Swindale 1954). Weathering of the micas produces various hydrous and expanded 2 : 1-type clay minerals; and eventually, under strong weathering, degradation of these micaceous (2 : 1-type) clays to kaolinitic (1 : 1-type) clays, and finally to secondary silica, occurs (Fieldes and Swindale, *loc. cit.*). It would be expected, therefore, that up to the stage where appreciable depletion of the interlayer K from these micaceous minterals has occurred, the equilibrium reaction

$$\text{difficultly-available interlayer or fixed K} \underset{\text{K fixation}}{\overset{\text{K release}}{\rightleftharpoons}} \text{exchangeable K}$$

would apply, and responses to added potassium would be unlikely. Such a mechanism would be of special value under grazing conditions where potassium is received in the urine patch at a very high rate but

very infrequently. At a later stage of weathering, when interlayer K has been further depleted, but the 2 : 1 clay lattice is still mainly intact, intermittent responses to potassium would be expected. This would occur because, although release of interlayer K is now inadequate to replenish fully the exchangeable K, the presence of 2 : 1-type clay permits the retention of added soluble K by fixation. At a still later stage, however, when the 2 : 1-type lattice has been largely broken down, the possibilities for K release or fixation no longer exist and the situation is therefore essentially similar to that obtaining in the intrazonal soils enumerated above: that is, the soils are permanently potassium responsive and responses are likely to be short-lived. In practice, this hypothetical weathering sequence is likely to be further complicated by the superimposing of leaching effects as the result of differing effective rainfalls, but it will nevertheless be apparent that the arguments advanced for the transient nature of potash responses on yellow-brown loams and other intrazonal soils cannot be applied to soils of the zonal groups without taking into account the fundamental differences in mineral composition.

It has been suggested that the main factors influencing the utilisation and conservation of potassium in the pasture soils of New Zealand are the rather intractable ones of the nature of the soil and the grazing behaviour of the animal. For practical purposes the soil must be taken as it comes, and it is unfortunate that much of the land climatically and topographically best suited to pastoral farming in New Zealand comes poorly supplied with reserves of potentially available potassium. The pattern of behaviour of the grazing animal is likewise not readily amenable to modification. There remains only the method of applying fertiliser potassium. Under the circumstances, the most suitable type of fertiliser would appear to be a slow-acting one which would release potassium over an extended period and so help to overcome the effects of luxury consumption by the plant and uneven distribution by the grazing animal. There is, as yet, no such fertiliser, and so the best that can be done is to apply soluble potassium fertilisers as efficiently as practicable. It would seem, therefore, that to maintain a high level of production and ensure vigorous growth of clover over an extended season, potassium should preferably be used in split dressings, perhaps in early spring (September) and in late summer (February) at rates determined by field trials. It is possible, also, that to obtain full benefit from the spring topdressing particularly it may be necessary to use nitrogen also. Very high rates of application of KCl appear to offer no advantage in increased production or duration of response, and as these are expensive, and may carry a risk of inducing hypomagnesaemia (Kemp 1958), they obviously cannot be recommended.

To summarise, it can be said that the potassium problem on the yellow-brown loams and similar soils is aggravated by an unfortunate alliance of several unfavourable factors—lack of potassium reserves in the soil, absence of potassium-fixing minerals, rapid uptake of potassium by the pature, uneven redistribution of urinary potassium by the animal, and the soluble nature of potassic fertilisers. From the immediate, practical point of view, the only way in which these difficulties can be partially ameliorated would seem to be by the use of small and frequent

applications of potassium; as a long-term solution the development of slow-acting and relatively insoluble fertilisers would appear to hold the most promise.

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THE EFFECTS OF FREQUENCY AND INTENSITY OF GRAZING ON THE PRODUCTIVITY OF A PASTURE OF SHORT-ROTATION RYEGRASS AND RED AND WHITE CLOVER

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Summary

An experiment is described in which the effects of four combinations of frequency and intensity of grazing* on the productivity and species yields of a pasture of short-rotation ryegrass and red and white clover were determined over a two-year period.

Annual herbage production was highest under long spelling.

Annual yields were also high under frequent light grazings, but were markedly less where the grazings were severe.

The growth of ryegrass was favoured by long spelling between grazings.

Red clover yielded equally well under long spelling or frequent grazings, provided the grazings were not severe.

White clover yields were highest under frequent grazing.

The most marked treatment effects on productivity were recorded in periods of low pasture production. Over the late summer and autumn, yields were highest where frequent hard grazings did not occur; but, during the winter, yields were highest in the grazing systems that favoured ryegrass growth or where losses of herbage through tissue decomposition were avoided.

The results are discussed in relation to the differential effect of temperature, light, and moisture supply on growth and botanical composition, as influenced by the different frequencies and intensities of grazing.

Introduction

Grassland farming in New Zealand is characterised by two factors which place it in a unique position when compared with other major grassland areas of the world. First, because of favourable climatic conditions, there is almost complete reliance on pasture herbage as the diet for livestock throughout most of the year, and second, the nitrogen for grass growth is obtained from the legumes, particularly white clover (*Trifolium repens* L.).

For these reasons, maximum sustained yield of herbage throughout the year is a primary objective of pasture management, while of equal significance is the need to maintain a balance between the grasses and legumes of the pasture mixture.

With these objectives in mind, the experiment reported here was designed to obtain information on the seasonal productivity of a pasture

* The term "intensity of grazing" is used to describe a second variable compared in this study; that of height of herbage remaining after each grazing.

of short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*), red clover (*Trifolium pratense* L.), and white clover (*Trifolium repens* L.) when subjected to different systems of grazing management. In view of the small amount of quantitative information published since the introduction of the hybrid short-rotation ryegrass into pastoral farming in New Zealand (Corkill 1945), it was considered that the results obtained would assist in formulating grazing management principles for this pasture type.

The effects of frequency and intensity of grazing or cutting on the yield and botanical composition of pastures has received only a small amount of attention by workers in New Zealand. In no experiment has the effect of both frequency and intensity of grazing or cutting been investigated.

Rigg and Askew (1931) comparing hay and aftermath yields with what the authors described as normal grazing management of a pasture containing perennial ryegrass, timothy, sweet vernal, cocksfoot, and suckling clover, obtained increased dry-matter yields from the hay and aftermath treatment. Similar results were obtained by Coop and Sears (1943) from a mowing trial of the effects of time of shutting and stage of growth for hay and silage of a pasture containing perennial ryegrass, cocksfoot, white clover, and a small amount of other species.

Hudson *et al.* (1933), from a series of mowing and grazing trials, showed that, for a perennial ryegrass and white clover pasture, an increase of approximately ten per cent in the annual dry-matter yield was obtained by halving the number of defoliations. In this experiment, however, the difference in yield between the two treatments was either negligible or in favour of the more frequent cuttings in periods of low production, i.e. the summer or winter periods. In addition, growth of white clover was encouraged under the more frequent cutting treatments. Sears (1949) obtained similar results from an experiment in which a complex mixture of pasture species was subjected to three different grazing treatments. Grazings at a herbage height of 8–10 in. down to 2 in. gave an increase of approximately 40% in annual dry-matter yield compared with close and continuous grazing. Intermediate results were obtained when the pasture was grazed at a herbage height of 3–4 in. down to 1 in. The main feature of this experiment was the dominance of short-rotation ryegrass in the most leniently grazed treatment and of white clover in the continuously grazed treatment. Hamblyn (1954) reported similar results in comparing the effects of spelling for various periods on d.m. yields and botanical composition of an old pasture composed of perennial ryegrass, cocksfoot, browntop, sweet vernal, Yorkshire fog, and white clover. Cutting at weekly intervals resulted in a reduction of approximately 50% in annual yield compared with cuttings at six-weekly intervals. Cuttings at intervals of two or three weeks gave intermediate results.

Indirect evidence is also available from a series of investigations carried out by Jacques *et al.* (1937a, 1937b, 1941, 1952) and designed primarily to study the effect of various management practices on the root development of some common New Zealand pasture plants. The

results obtained showed that frequent and intensive defoliation reduced shoot and root development.

The results obtained in New Zealand are in general agreement with those obtained by a large number of workers throughout the world. This volume of literature will not be reviewed in detail in this paper but some general comments are made.

Many of the investigations have shown that increased frequency of grazing or cutting of pasture results in decreased dry-matter yields. Some examples of this result are those obtained by Stapledon (1924) and Jones, L. (1933, 1934) working in the United Kingdom with cocksfoot-dominant pastures and cocksfoot and tall fescue respectively; by Dotzenko and Ahlgren (1950, 1951) working in the U.S. with alfalfa-bromegrass pastures; by Wilsie *et al.* (1937, 1940) working in Hawaii with alfalfa and Napier grass; by Davies and Sim (1931) and Richardson (1932) working in South Australia with *Danthonia penicillata* and *Festuca bromoides,* and perennial ryegrass and cocksfoot-dominaant pastures respectively; by Klapp (1942) working in Germany with cocksfoot; by Lander (1942) working in the Punjab with *Pennisetum cenchroides* and *Andropogon annulatum;* and by Louw (1938) working in South Africa with species such as *Chloris gayana* and *Digitaria pentzii.* These examples illustrate that many workers have obtained a similar general result in a large number of different environments for a wide range of erect-growing species. Where different results have been obtained, the dominant species were usually of a prostrate or stoloniferous habit as has been shown by Davies and Sim (1931) for *Erodium botrys,* Jones, L. (1933, 1934) for white clover, bent, and rough-stalked meadow grass, and Ahlgren (1938), in the U.S. for Kentucky bluegrass.

Similar results have also been obtained where the effect of intensity of grazing or cutting on dry-matter yields has been determined. With erect-growing species, intensive defoliation is associated with lower yields as has been shown by Stapledon (1924) and by Jones, M. (1933) working with perennial ryegrass in England, Hildebrand and Harrison (1939) working with alfalfa in the U.S., and Weinmann (1948) working with *Trachypogon plumosus* and *Tristachya hispida* in South Africa. However, when prostrate-growing species have been examined, intensive defoliation has usually been associated with highest yields. An example of this is the work that has been carried out on Kentucky bluegrass in many different locations in the U.S. as shown by Graber (1933), Mortimer and Ahlgren (1936), Robinson *et al.* (1947, 1952), and Kennedy (1950).

From the above paragraphs the generalisation can be made that the more frequently and more intensively a pasture is grazed the more will the prostrate-growing species be encouraged and the more will the erect-growing species be discouraged. However, there are many exceptions to this generalisation as is instanced by the work of Kennedy (1950), who showed that increased frequency of clipping of pastures in which the stoloniferous species Kentucky bluegrass was the dominant component resulted in decreased yields, and by Tesar and Ahlgren (1950) who demonstrated a similar result for the stoloniferous species Ladino clover in the U.S.

The above results have usually been explained in terms of the quantity of photosynthetic tissue removed by the defoliation treatment and its effect on subsequent regrowth (Brougham 1956a, Davidson and Donald 1958), the amount of light received by the components of the pasture as influenced by the defoliation treatment (Blackman 1938, Donald 1956), or the effect of defoliation on the amount of stored reserves remaining for regrowth. An extensive review of literature on this last factor has recently been published (Troughton 1957).

Other factors have been considered and amongst these are mechanical damage to growing points through grazing (Branson 1953, Scott 1956), the ratio of fertile to vegetative tillers at grazing (Branson 1953), the inability of severely defoliated plants to utilise soil water (Jantii and Kramer 1956), and the induced unavailability of soil nitrogen through drying out of soil under intensive defoliation (Mitchell 1957). Treading effects on pasture production have been reviewed by Edmond (1958b).

EXPERIMENTAL

The experiment was carried out at the Palmerston North station of the Grasslands Division on Manawatu silt loam. For two years before ploughing in February 1954, the area had been in spaced plants of *Lolium* spp.

In late March 1954, the experimental area was sown to a mixture of short-rotation ryegrass at 20 lb per acre, red clover at 4 lb per acre, and white clover at 3 lb per acre. At sowing, superphosphate was applied at the rate of 3 cwt per acre and during the experiment annual dressings were made at this rate. 4 lb of 100 per cent DDT per acre was added to the 1956 dressing.

By September 1955, all species in the pasture were successfully established and first grazings of the following four treatments began on 24 September 1955.

(1) Grazings when the herbage reached a height of 3-4 in. down to a height of 1 in. (3-1).

(2) Grazings when the herbage reached a height of 6-9 in. down to a height of 1 in. (9-1).

(3) Grazings when the herbage reached a height of 7-8 in. down to a height of 3-4 in. (7-3).

(4) Grazings when the herbage reached a height of 9-12 in. down to a height of 3-4 in. (12-3).

These four treatments were determined with reference to the growth curves of the above pasture type (Brougham 1955). In that experiment the rate of regrowth following defoliation was shown to follow a sigmoid curve of growth. In another experiment (Brougham 1956a) it was shown that the initial phase of growth continued until there was enough green leaf present to intercept almost all the incident light. Thereafter growth was at a constant maximum rate until factors limiting to growth became operative.

In view of this background information, an attempt was made in treatments 3 and 4 to leave a considerable amount of green tissue after grazing while the remaining two treatments were grazed to 1 in. at each grazing so that most of the photosynthetic tissue was removed. Within these two groups one treatment was grazed frequently and the other spelled for a longer period.

The four treatments were replicated five times in randomised blocks. The 20 plots, each 1 ch $\times$ $\frac{1}{2}$ ch, were fenced so that each could be grazed separately. Mature wethers were used for grazing and replicate plots were grazed at the same time for each grazing.

To avoid fertility transference on or off the experimental area, the wethers were pre-fed on pastures of similar composition. For treatments 1 and 2, where the pastures were hard grazed at each grazing, and consequently the sheep were comparatively hungry when removed, an attempt was made to ensure that the animals had been at a similar level of feeding before the grazings commenced. For the lightly grazed treatments (7–3 and 12–3), where a considerable amount of herbage was left after each grazing, care was taken to ensure that the animals had been adequately fed before being used.

Stock numbers at each grazing were also adjusted so that grazings could be completed in 24 or 48 hours depending on the bulk of feed present. In this way fertility transference by the animal was minimised (Goodall 1951, Sears 1953b). However, in treatment 3 where a high stocking rate was required to graze each plot uniformly, grazings were completed in only a few hours over the winter period. Because of this, some fertility may have been transferred from these plots. An attempt was made to avoid some of this transference by alternating grazings from day to night over this period.

At times towards the end of the grazing period of the 7–3 and 12–3 treatments, a sickle-bar mower was used to top uneaten patches of herbage. This was done to ensure that regrowth was uniform.

At each grazing, two 10 ft $\times$ 4 ft frame enclosures were placed at random within each plot. At the end of each grazing the enclosed herbage was cut by scythe or hand-shears down to the height of the surrounding grazed pasture as described by Sears (1951). The cut herbage was then weighed and sub-samples were taken for dry-matter determinations and botanical analyses. The latter were hand separated and composition determined on a dry-weight basis. All herbage was oven dried at 160–180°F.

At intervals throughout the experiment, determinations of ryegrass tiller numbers per unit area were made. For these, 35 cores of approximately 2 in. diameter were taken at random from each plot, and the number of ryegrass tillers present in each core was determined. The method has been recently described (Mitchell and Glenday 1958). From calculations of the percentage of cores containing ryegrass, a measure of tiller dispersion was obtained.

During the summer of 1956-57, soil-moisture determinations were made in the 3–1 and 7–3 treatments. The plots of these treatments

were sampled using a corer of 1 in. diameter to four depths below ground level, viz, $\frac{1}{2}$–3 in., 3–6 in., 6–9 in., and 9–12 in. Six samples of each layer were taken at random from each replicate and weighed. These were then oven dried at 100°c until weights became constant. The results are presented as percentage moisture of dried soil.

The experiment ended on 6 December 1957.

RESULTS

Total Dry-matter Production and Species Yields

Yield data, unless otherwise stated, are expressed in lb dry matter (d.m.) per acre.

(*a*) *Annual Yields*

Table 1 shows total and species d.m. yields for the four treatments, together with the number of grazings in each treatment for each of the twelve-month periods. Also shown are the least significant differences at the 5% level. Because of the small amounts of other grasses and other species in each treatment, statistical analyses were not performed for these components.

TABLE 1. ANNUAL YIELDS

Period	Treatment	No. of Grazings	Total D.M. Yield (lb/acre)	Species Yields				
				Ryegrass	Red Clover	White Clover	Other Grasses	Other Species
2/12/55–27/11/56	3–1	17	14,410	11,130	370	2510	220	180
	9–1	10	17,560	13,060	1820	2480	20	180
	7–3	18	17,100	11,630	2760	2560	90	60
	12–3	12	17,070	11,970	3070	1990	tr	40
	Sig. diff. at 5% level		1,075	1,250	575	575	—	—
27/11/56–6/12/57	3–1	12	11,230	5,570	70	4620	620	350
	9–1	10	16,190	12,980	310	2510	160	230
	7–3	15	14,280	9,580	630	3930	50	90
	12–3	11	14,270	11,750	560	1860	30	70
	Sig. diff. at 5% level		1,475	2,225	200	1175	—	—

The following are the significant features of Table 1:

(1) The pastures under frequent hard grazings (3–1) yielded less total d.m. than the other treatments. In the first year this was due to a marked reduction in red-clover growth and in the second year to reductions in both ryegrass and red-clover growth.

(2) Total d.m. yields obtained from the pastures under both systems of lax grazing did not differ significantly although in the

second year some differences in the yields of component species were shown. In the 7–3 treatment a reduced yield of ryegrass was balanced by an increase in the growth of white clover.

(3) Total d.m. yields from the 9–1 treatment were similar to the yields obtained from the laxly grazed treatments in the first year. Although the growth of ryegrass was greater in this treatment, it was associated with decreased growth of red clover. In the second year a higher total d.m. yield was obtained from the 9–1 treatment because of an increase in ryegrass growth.

(4) Ryegrass yields were highest under long spelling.

(5) White-clover yields were highest under frequent grazings.

(6) Red-clover yields were markedly reduced under hard grazings.

(7) Yields obtained from species other than those sown were highest in the hard-grazed pastures.

(*b*) *Seasonal Yields*

The results presented in Table 2 show total and species d.m. yields for each season. Also shown are the number of grazings in each season for each treatment and the least significant differences at the 5% level.

The following comparisons are of interest:

(1) For each season except the winter of 1956, the 3–1 treatment yielded less total d.m. than the 9–1 or 7–3 treatments. In the summer and autumn of 1956 this was due to reduced growth of clover and in particular red clover, whereas for the remaining seasons the differences were due mainly to reduced growth of ryegrass. During the winter of 1956, the 3–1 treatment outyielded the 7–3 treatment but did not differ significantly in yield from the 9–1 treatment.

(2) In comparing the 3–1 and 12–3 treatments, consideration must be given to the changing botanical composition in both treatments that influenced total d.m. yields in the first three seasons of the experiment. In the first summer a marked reduction in red-clover growth in the 3–1 treatment was associated with a reduction in total d.m. yield but in the following autumn this was balanced by a reduction in ryegrass yield in the 12-3 treatment so that no significant differences between total yields were recorded. In the winter of 1956 the 3–1 treatment outyielded the 12–3 treatment because of increased ryegrass growth. For the remaining five seasons reduced growth of ryegrass in the 3–1 treatment was associated with reductions in total d.m. yield. These reductions in ryegrass yield were of sufficient magnitude to offset increases in the growth of white clover.

(3) Comparisons of the 9–1 and the laxly grazed treatments (7–3 and 12–3) show that in the first summer a reduction in red-clover growth in the 9–1 treatment was sufficient to cause a small but significant decrease in total d.m. yield compared with the 7–3 treatment. In the other comparison with the 12–3 treatment, this reduction in red

Period	Treatment	No. of Grazings	Total D.M. Yield (lb/acre)	Ryegrass	Red Clover	White Clover	Other Grasses	Other Species
Spring 28/9/55-2/12/55	3–1	5	5050	3000	340	1300	320	90
	9–1	3	5500	3200	560	1480	200	60
	7–3	4	4850	2550	950	1150	160	40
	12–3	3	4750	2900	1040	720	70	20
	Sig. diff. at 5% level		500	450	360	290	—	—
Summer 2/12/55-29/2/56	3–1	5	4700	3080	220	1180	110	110
	9–1	2	5800	2920	1300	1480	tr	100
	7–3	5	6660	3000	1980	1560	80	40
	12–3	3	6160	2860	2260	1020	tr	20
	Sig. diff. at 5% level		600	640	460	390	—	—
Autumn 29/2/56-1/6/56	3–1	3	2240	1580	100	540	—	20
	9–1	2	2820	1740	440	580	—	60
	7–3	3	2760	1680	580	500	—	tr
	12–3	2	2340	1120	700	520	—	tr
	Sig. diff. at 5% level		280	360	220	200	—	—
Winter 1/6/56-31/8/56	3–1	3	2420	2220	20	150	20	10
	9–1	2	2540	2400	20	100	20	tr
	7–3	3	1480	1400	20	60	—	—
	12–3	2	1920	1840	20	50	—	10
	Sig. diff. at 5% level		360	400	—	40	—	—
Spring 31/8/56-27/11/56	3–1	6	5050	4250	30	640	90	40
	9–1	4	6400	6000	60	320	tr	20
	7–3	7	6200	5550	180	440	10	20
	12–3	5	6650	6150	90	400	tr	10
	Sig. diff. at 5% level		400	520	—	180	—	—
Summer 27/11/56-28/2/57	3–1	3	3800	2250	20	1400	10	120
	9–1	2	5300	3800	200	1120	60	120
	7–3	4	5000	3100	360	1450	30	60
	12–3	3	5000	3850	340	800	—	10
	Sig. diff. at 5% level		750	950	150	600	—	—
Autumn 28/2/57-1/6/57	3–1	2	1440	160	20	1200	—	60
	9–1	2	2400	1600	80	680	tr	40
	7–3	3	2300	700	220	1360	—	20
	12–3	2	1680	800	200	640	10	30
	Sig. diff. at 5% level		200	380	70	320	—	—
Winter 1/6/57-31/8/57	3–1	2	1140	560	10	480	50	40
	9–1	2	2240	2080	10	130	tr	20
	7–3	2	1480	1280	10	180	10	tr
	12–3	1	2040	1900	10	120	—	10
	Sig. diff. at 5% level		280	320	—	130	—	—
Spring 31/8/57-6/12/57	3–1	5	4850	2600	20	1540	560	130
	9–1	4	6250	5500	20	580	100	50
	7–3	6	5500	4500	40	940	10	10
	12–3	5	5550	5200	10	300	20	20
	Sig. diff. at 5% level		760	1050	—	360	—	—

clover was offset by a reduced yield of white clover in the 12–3 treatment. In both winters reduced growth of ryegrass in the 7–3 treatment was associated with reductions in total d.m. yields compared with the 9–1 treatment, and a similar pattern is shown for both autumn periods and the first winter in the 12–3 and 9–1 comparison. Some other small botanical changes are evident in the 7–3 and 9–1 comparison in the autumn and spring of 1957 but in both cases reduced ryegrass growth in the 7–3 treatment was offset by increases in clover growth.

(4) In the comparison between the laxly grazed treatments (7–3 and 12–3) no significant differences in total d.m. yield were recorded in the spring and summer periods. However, except for the first spring, the 7–3 treatment induced more white-clover growth. In the first summer this increase was not sufficient to influence total d.m. yields, and in the spring and summer of the second year it was offset by increased ryegrass growth in the 12–3 treatment. In the first autumn the 7–3 treatment yielded more than the 12–3 treatment because of increased ryegrass growth, and in the second autumn a similar difference was recorded because of reduced white-clover growth in the 12–3 treatment. In both winters these differences were reversed because of reduced ryegrass growth in the 7–3 treatment.

The above results are illustrated graphically in Fig. 1 where the average daily total and species dry-matter increments of each treatment in lb per acre are shown for each month of the experiment. Each point on the graph was obtained by dividing the species and total d.m. yields determined at each grazing by the number of days of growth between it and the preceding grazing. The figures so obtained were then averaged on a monthly basis. Because of the small number of grazings in some seasons and the fact that the growth rate between grazings is not constant (Brougham 1956a), the figures can only be regarded as an approximation.

The trends are evident and in general conform to the pattern shown in Table 2. Salient features are:

(1) The pastures were ryegrass dominant in winter, spring, and early summer, and clover dominant in the late summer and autumn.

(2) The highest total daily growth rate occurred in the spring (November-December) and reached a value of 80–100 lb d.m. per acre.

(3) The lowest yields were recorded in the winter. However, except for the 3–1 treatment, the total daily growth rate was never much lower than 15–20 lb d.m. per acre.

(4) A gradual decline in total growth rate is evident from late spring to winter and only a slight slowing-up is shown over the autumn period. This was more pronounced in the second year.

(5) A rapid increase in total growth rate in the late winter and early spring (August-September) occurred.

(6) Increased clover dominance in the autumn (e.g. the 7–3 treatment) was associated with decreased total d.m. yields over the winter.

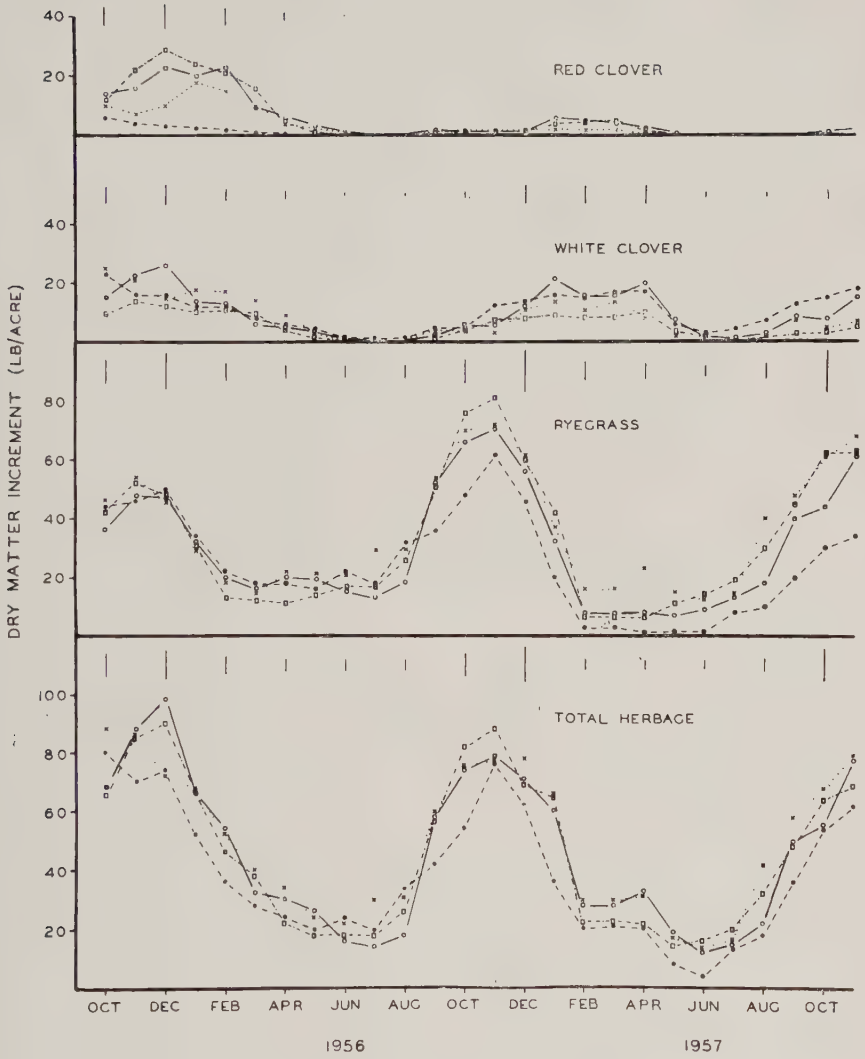

Fig. 1.—Average daily total and species dry-matter increments of each treatment for each month of the experiment. Average standard errors of estimate are also shown.

- - - - - - 3–1 treatment; x . . . . . x 9 1 treatment;
o ———— o 7–3 treatment; - . - . - . - . - 12–3 treatment.

Tiller Numbers

Table 3 shows tiller numbers per sq. ft for the four treatments taken on six different sampling dates during the latter half of the experiment. The least significanct differences at the 5% level of probability are also shown.

TABLE 3. Tiller Numbers per Square Foot

Treatment	Date of Sampling					
	4/7/56	28/9/56	27/2/57	26/4/57	2/8/57	12/11/57
3–1	300	260	70	36	72	120
9–1	290	170	160	116	188	280
7–3	260	240	170	112	204	170
12–3	200	150	170	76	160	180
Sig. diff. at 5% level	—	60	100	40	40	90

The major points of interest in this table are:

(1) Apart from the first two samplings, tiller numbers per unit area in the 3–1 treatment were consistently lower than the numbers in the other three treatments.

(2) High tiller populations were consistently recorded in both the 7–3 and 9–1 treatments and only minor differences were obtained between these treatments except for the sampling in September 1956 when the 7–3 treatment had a higher number than the 9–1 treatment and the last sampling when the 9–1 treatment had a higher number than the 7–3 treatment.

(3) Numbers in the 12–3 treatment were generally lower than the 7–3 or 9–1 treatments although the differences only reached significant level on three sampling dates. In September 1956 and August 1957, numbers were significantly lower than those obtained from the 7–3 treatment, and in April 1957 they were significantly lower than those in the 9–1 treatment.

(4) At the last count the 9–1 treatment contained more tillers per unit area than any of the other treatments.

Percentage Occurrence of Tillers

The results presented in Table 4 show the percentage of cores containing ryegrass tillers at each sampling date. This index when related to tiller numbers can be used as a measure of tiller dispersion or clumpiness.

TABLE 4. Percentage Occurrence of Ryegrass Tillers

Treatment	Date of Sampling					
	4/7/56	28/9/56	27/2/57	26/4/57	2/8/57	12/11/57
3–1	60.0	63.2	37.4	21.8	38.6	49.2
9–1	34.4	44.8	56.8	64.6	80.4	85.2
7–3	49.0	60.6	59.6	61.6	75.6	75.8
12–3	38.4	41.4	52.2	61.4	72.8	72.0
Sig. diff. at 5% level	16.4	11.0	15.6	11.3	11.1	15.1

The important features of this table are:

(1) For the first two counts a higher percentage occurrence was shown in the frequently grazed treatments (3–1 and 7-3) although in the first count significance was reached only in the 3–1 treatment.

(2) For the remainder of the samplings the percentage occurrence in the 3–1 treatment was lower than in all other treatments. Differences between the remaining treatments did not reach significance.

Soil Moisture Determinations

Table 5 shows the results of soil-moisture determinations for the frequently grazed treatments (3–1 and 7–3) made on 22 January 1957 immediately after grazing.

TABLE 5. SOIL MOISTURE (PERCENTAGE OF DRY SOIL)

Depth (in.)	3–1	7–3
½– 3	14.5	18.0
3 – 6	16.4	17.9
6 – 9	17.5	18.8
9 –12	17.4	18.6
S.E.	±0.46	±0.45

(1) At all levels the soil of the 3–1 treatment was significantly drier than that of the 7–3 treatment (at the lowest level, the difference just failed to reach significance, $p = 0.07$).

(2) The difference in favour of the 7–3 treatment was significantly greater in the surface layer.

DISCUSSION

The results presented show definite relationships between the growth of the species in the pastures and the different frequencies and intensities of grazing. These relationships vary with season but follow a logical pattern determined by species tolerance to the grazing systems and by the interaction of the various weather factors on growth. In general they agree with results obtained from the large number of previous investigations.

The grass under study in this experiment is characterised by a relatively erect habit of growth and, as has been shown, it is more productive under long spelling than under frequent grazing. Its inability to withstand hard grazings, particularly during the summer and autumn of the second year, is shown in Table 2. This is in direct contrast to the ability of perennial ryegrass to withstand severe defoliation (Klapp 1937).

Red clover has also an erect growth habit and under spelling followed by hard grazing all photosynthetic tissue is removed. Repeated intensive defoliations result in death of plants. This is shown in the

results obtained for the 3–1 and 9–1 treatments where a marked reduction in red clover yield has been recorded (Table 1). Highest yields were obtained under both systems of lax grazing.

White clover, on the other hand, is a prostrate-growing stoloniferous species and in contrast to the other sown species of the pasture the highest production was obtained from the frequently grazed pastures. Under long spelling the reduced growth of white clover is attributed to overshading by the associated grass growth which resulted in a decrease in the amount of light energy available for the growth of this species (Blackman 1938, Donald 1956, Brougham 1957).

The dominant species of the pastures during the winter, spring and early summer was short-rotation ryegrass but during the late summer and autumn the botanical composition of the pastures changed markedly to clover dominance. This pattern of growth is in agreement with that obtained in a previous experiment (Brougham 1959) for a pasture of similar composition but under irrigation. The explanation offered for the changes in composition in that experiment in terms of species temperature and light tolerance would also apply here.

In the previous experiment the daily dry-matter increment of total growth reached a peak of 120 lb per acre in late December, and fell to 10 lb per acre in early July. The estimated potential yield of the pasture was 22,000 lb d.m. per acre. In the present experiment the calculated daily dry matter increment and annual total yield was generally 20-30% lower except in the winter when a daily increment of 15–20 lb of d.m. per acre was recorded. Compared with the potential yield of the previous experiment, this reduction is attributable to a number of factors which would include incomplete utilisation of light energy at different times of the year (Brougham 1956a, 1958), losses of herbage through tissue decomposition (Brougham 1956b), plant damage by treading (Edmond 1958a), and low available moisture during the summer and early autumn.

The differences between the seasonal growth patterns produced by the different treatments (Table 2) were determined by species tolerance to the grazing systems and the modified action of weather factors on growth because of differences in herbage cover and in botanical composition. During the spring, when moisture was adequate and temperatures favoured ryegrass growth, the pastures under both systems of long spelling and the frequently lax-grazed pastures were dominantly ryegrass. The height and ground cover attained by the herbage in these treatments would have been sufficient to utilise most of the available light energy during this period so that rate of ryegrass growth was high but overshading of white clover occurred. By comparison, the herbage in the frequently hard-grazed pastures was at all times relatively short and provided better light conditions for the growth of white clover. An increased percentage of white clover resulted but lower total yields were obtained.

During the summer and autumn, climatic conditions generally favoured the growth of the clover species but the availability of soil

moisture and the amount of light received by the components of the pastures was influenced by the different systems of grazing. The results presented in Table 5 show that where a cover of herbage was maintained, the amount of soil moisture available for growth was significantly higher. This would result in an increased availability of soil nitrogen in the top layers of soil (Mitchell 1957). Both factors favoured growth in the laxly grazed treatments. Although measurements were not made in the other long-spell treatment, it is probable that the ground surface was covered by herbage for most of the time because of the small number of grazings carried out. Consequently growth would also be favoured by increased availability of soil moisture and nitrogen.

In the frequently hard-grazed pastures, the drier conditions restricted growth, and, as is shown in Tables 2 and 3, widespread death of ryegrass occurred in the second summer and autumn. It is also possible that even where some soil moisture was available, the severely defoliated plants may have been unable to utilise the water for growth (Jantii and Kramer 1956).

The results obtained (Tables 2 and 3) also suggest that in the treatments favouring growth of red clover during the summer (the less intensively grazed treatments and in particular the 12–3 treatment), interspecies competition for light occurred. The base shading of ryegrass plants that followed was associated with a reduced rate of tillering (Mitchell and Coles 1955) and a reduction in ryegrass growth in the next autumn. A similar pattern is evident during the autumn (Table 2), where increased white clover growth was associated with a reduced growth of ryegrass in the following winter. This was particularly evident in the frequently but less intensively grazed pastures in the second autumn and winter.

The pattern of growth in the winter when the hard-grazed treatments outyielded the less intensively grazed treatments in the first year, and the 9–1 treatment alone outyielded these treatments in the second year, is more difficult to explain, although a partial explanation has been offered above. It is considered that some of the losses were also attributable to a limitation in the measurement technique used. The measured growth in both less intensively grazed treatments was that above a 3–4 in. level. Previous work (Brougham 1956b) showed that considerable losses of herbage occur through tissue decomposition below this level during the winter. This was not accounted for by the measurement technique used nor was the new growth that occurred below this level.

The relatively high yield obtained from the frequently hard-grazed treatment in the first winter can be explained in terms of light interception by the foliage. Over the winter the angle of incidence of sunlight is small and light intensity is low. The amount of foliage required to intercept and utilise all available light is therefore small (Brougham 1958) and it is probable that a relatively high growth rate was maintained throughout most of the winter in the 3–1 treatment. Losses of herbage through tissue decomposition would also be negligible.

In the second winter, yields from this treatment were low because of death of ryegrass in the preceding summer (Table 3).

Marked seasonal fluctuations in ryegrass tiller number were also obtained (Table 3) and these are attributed to the influence of temperature on rate of tillering (Mitchell 1954), base shading from associated clover growth, and floral initiation. The lower counts obtained in all treatments in the autumn sampling were obtained at a time of the year when temperatures favoured clover growth and reproductive growth in ryegrass had occurred. Rate of tillering would therefore be relatively low and not sufficient to compensate for losses through grazing, treading, lack of soil water, or high temperatures.

The consistently lower counts in the 3–1 treatments after the spring of 1956 and the lowered percentage occurrence (Table 4) were due to death of ryegrass plants. The low numbers in the 12–3 treatment at most sampling dates compared with the 7–3 and 9–1 treatments were probably determined by a greater degree of base-shading.

The results show that, for this pasture type growing in localities with a similar climatic pattern, high annual yields of herbage can be obtained from management systems in which long spelling between grazings is practised. However, yields of equal magnitude can be obtained under frequent grazing provided a cover of herbage is left after each grazing. In practice this latter system of management has some advantages. At all times of the year the herbage eaten by the grazing animals is leafy and provides a high-protein diet. By comparison, the feed offered to the animals in the long-spelled systems of management is at times less palatable because of an increased production of seedhead over the summer and a higher proportion of stem tissue. The greater number of grazings obtained from the more frequent grazing system is also desirable, particularly in periods of feed shortage when an increased frequency of grazing is often enforced because of prevailing weather conditions. In this respect the fact that approximately 1,000 lb of d.m. per acre is present below the 3–4 in. level is a desirable feature. However, it is probable that no single system of management is satisfactory, as from the results presented the productivity of this pasture type under different management systems changes with season. Rather it is considered that the most favourable management system would be one in which the desirable features of the less intensively grazed pastures over the spring, summer, and early autumn are combined with hard grazings over the late autumn. These grazings would bring about a rapid change in botanical composition to grass dominance so that high yields are obtained over the winter.

In conclusion, the experiment as reported was designed to determine the influence of various frequencies and intensities of grazing on the productivity of a pasture of short-rotation ryegrass and red and white clover, and to attempt to determine factors that influence the growth of the species and changes in botanical composition. In view of the results obtained and the many parallel field observations made, the need for wide-scale field experiments covering both pasture and animal productivity reactions to differential frequencies and intensities of grazing is obvious.

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PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XIX. THE SUSCEPTIBILITY OF NEW ZEALAND WHITE RABBITS TO FACIAL-ECZEMA LIVER DAMAGE

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(*Received for publication, 25 September 1959*)

Summary

White rabbits fed on grass containing the facial-eczema hepatotoxin developed liver lesions similar to those seen in guinea pigs and lambs, but oedema of the extrahepatic ducts was more frequently seen. Similar lesions were produced in rabbits by feeding spores of *Sporidesmium bakeri* and ether extracts of toxic grass and of *S. bakeri* cultures. The rabbit appears more susceptible than the guinea pig to the facial-eczema toxin.

Introduction

Studies leading to the practical use of guinea pigs as test animals have been described by Evans *et al.* (1957) and Perrin (1957). Rabbits were also tried, without success, by Cunningham (private communication), but only in paddock grazing experiments. Availability of larger amounts of toxic dried grass in 1957 led to further trials with rabbits, for species comparison purposes and because the rabbit, if susceptible to the hepatotoxin, might be a useful animal for experiments requiring bleeding or surgery. This paper records experiments which indicate not only that the New Zealand white rabbit is susceptible, but also that it is more sensitive than the guinea pig to this hepatotoxin.

Experimental

Dried, ground grass samples were mixed with water before feeding in the manner described by Perrin (1957). Later, dried grass was fed unground. For this purpose a cylindrical feed container was made from a tin, length 4 in., diameter 3 in., closed at both ends, but with an opening 2 in. wide cut along the full length, and held to the cage by a wire loop. The curved sides of this container prevented the spilling of grass which had previously prevented feeding of unmilled samples.

Ether extracts of grass were made in a copper extractor similar in design to the glass apparatus described by Letham *et al.* (1957). The more concentrated "3000 fraction" was prepared by the procedure of White (1958). For feeding, these extracts were dispersed on ground non-toxic grass or on a meal mixture, consisting mainly of barley and wheat meal, sieved to remove dust. It was not necessary to mix this meal with water before feeding to either rabbits or guinea pigs.

Body weight was recorded three times a week, and these weights were used to calculate consumption per kilogramme of body weight for each day. All livers were examined by Mr D. C. Dodd who has separately described the pathology of facial eczema in rabbits (Dodd, in preparation).

The extract of *Sporidesmium bakeri* was prepared from a sporing 7-day culture, macerated in the potato carrot medium on which it was grown. This macerate was extracted with ether in a continuous liquid/liquid extractor for 8 hours, and the extract concentrated in a vacuum rotary evaporator.

Results and Discussion

The results are summarised in Table 1 and Fig. 1. The first rabbit fed (Rabbit 1) consumed daily between 70 g and 80 g dried grass per kilogramme of body weight over the first 9 days. Its intake then dropped steeply to 11 g on the 12th day and remained about this level until the rabbit was killed, moribund, on the 15th day (Fig. 1 (i)). The liver did not show the mottled appearance common in guinea pig and sheep livers affected with facial eczema. Instead there was considerable oedema of the walls of the extrahepatic bile ducts, extending along the full length of the cystic and common ducts, but not into the gall bladder. These ducts were patent. Sectioning the liver revealed similar thickening of the walls of the larger intrahepatic ducts. Histological examination showed lesions essentially the same as those seen in sheep and guinea pigs affected by the facial-eczema poison (Dodd, in preparation).

The grass sample used for Rabbit 1 had previously been fed to guinea pigs at a level of 200 g per kg initial body weight for 35 days, and produced liver damage graded as "severe" under the system described by Perrin (1957). This grass was fed to three other rabbits, and other samples of various levels of toxicity (as indicated by guinea pig feeding) were similarly fed. The results are given in Table 1 and Fig. 1.

In addition to these samples, a grass graded as mild was fed as part of another experiment to five rabbits killed at seven days and to five killed at 14 days. Intake levels ranged from 55 to 72 g per kg body weight, none of these rabbits showing a fall in intake. The livers of two of the 14-day group showed a slight oedema of the larger bile ducts. The rest appeared normal but histological examination revealed facial-eczema lesions in three of the seven-day group, and in all except one of the 14-day group.

Three aspects of these results are of especial interest:

1. The greater susceptibility of the rabbit, compared with the guinea pig, to the toxic factor.
2. The striking fall in consumption shown by most affected rabbits.
3. Unusual features of the liver lesions in the rabbit, particularly the lesions in the extrahepatic ducts.

Comparative Susceptibility of Rabbits and Guinea Pigs

The results indicate that the rabbit is more susceptible than the ginuea pig to the facial-eczema hepatotoxin. Normally, guinea pigs survive for the full 5-week period which has been adopted for testing toxic grasses, even on "severe" samples, which produce grossly enlarged, fibrous, and often bile-stained livers. The amount of grass

TABLE 1. TOXICITY OF GRASS AND EXTRACTS

Rabbit No.	Sample and Toxicity Grading	Body-weight Change (kg)	Intake		Days to Fall in Intake	Days to Killing	Liver Weight (g/kg b.w.)	Liver Lesions	
			Total (g)	Mean Daily (g/kg b.w.)				Macro.	Hist.
1	Grass A, severe	1.05–0.95	890	56	9	15	31	+	+
2	Grass A, severe	0.98–0.93	580	57	7	10	33	+	++
3	Grass A, severe	0.46–0.59	820	80	19	21	31	+	+
4	Grass A, severe	1.11–1.07	830	68	8	11	29	+	
5	Grass B, mod. sev.	0.70–0.57	620	56	13	16	37	+	+
6	Grass B, mod. sev.	0.70–0.59	650	62	15	17	24	+	++
7	Grass C, mod.	0.94–1.07	1650	45	20	35	30	+	+
8	Grass D, mod.	0.48–0.40	230	61	7	8	50	+	
9	Grass D, mod.	0.75–1.11	1700	66	—	35	27	+	+
10	Grass D, mod.	0.72–1.10	1840	66	—	29	28	?	+
11	Grass D, mod.	0.68–0.99	1070	72	—	18	31	—	—
12	Grass E, mod.	0.94–0.79	970	45	22	24	25	+	+
13	Grass E, mod.	0.96–0.84	690	48	16	19	32	+	++
14	Grass E, mod.	1.02–0.88	610	40	16	18	30	+	++
15	Grass E, mod.	0.91–0.72	320	40	10	11 (died)	27	+	++
X1	Ether extract	0.56–0.62	500	90	8	9	37	+	+
X2	Ether extract	0.72–0.67	630	84	8	10	48	+	
X3	Ether extract	0.54–0.45	470	70	6	11	29	+	+
X4	"3000" fraction	0.57–0.52	1080	280	6	8	37	+	+

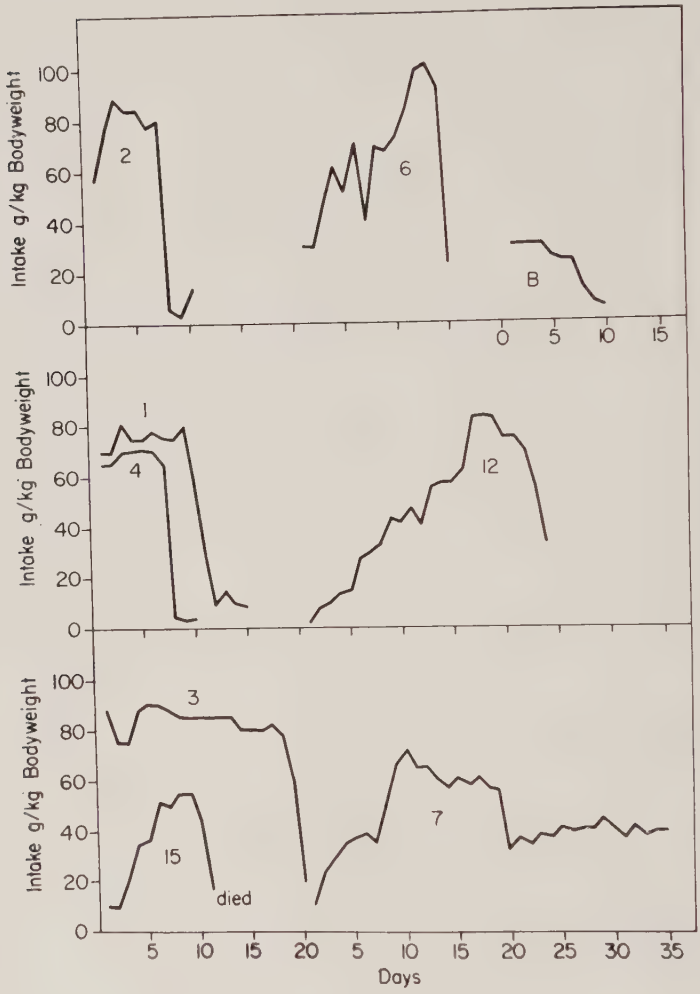

Fig. 1.—Intake of affected rabbits.

eaten by these guinea pigs is about 1 kg—150–200 g/kg body weight per day, or 5–7 kg/kg body weight in 35 days. The amount consumed by Rabbit 3, the slowest of those on a "severe" grass to cease eating, was only 820 g or 1.6 kg/kg body weight. The mean consumption of the other three rabbits on this "severe" sample was only 0.72 kg/kg body weight.

Similarly, with grass samples graded as moderate by guinea pig feeding, rabbits have shown evidence of toxicity by a fall in consumption (which may eventually have led to death by starvation) in times much less than the 35-day feeding period used for guinea pigs. For example, 15 guinea pigs fed on grass E all survived to 35 days, and all except one showed "mild to moderate" or "moderate" liver damage.

The four rabbits on this sample abruptly dropped their intake by 50% or more after 10, 16, 16, and 24 days. The amount eaten by these guinea pigs was lower than usual owing to the nature of the sample, varying from 2.6 to 3.2 kg/kg body weight.

In considering these results, it must be remembered that if the guinea pigs had been killed earlier many would have shown liver damage, but from general experience in such feeding experiments macroscopic liver damage is rarely seen in guinea pigs at 10–16 days, on the consumption levels recorded for the rabbits. For strict comparison of susceptibility between the two species an experiment involving feeding at the same level for the same time is required.

Fall in Consumption in Affected Rabbits

Intake curves for eight of the rabbits are shown in Fig. 1. All rabbits on the "severe" sample showed a marked drop in consumption, to less than 50% of their previous intake, in times varying from 7 to 19 days. Similar depression of intake occurred with six of the eight affected animals on "moderate" samples. Some of the rabbits were moribund after two or three days refusal of feed (including fresh grass), others were killed before this stage was reached to ensure examination of the liver without post-mortem changes. Rabbit 7 is of particular interest. It showed a drop in consumption from 66 g/kg to 33 g on the 20th day, but continued at about 40 g until it was killed at 35 days. In addition to oedema of the extrahepatic ducts the liver showed the surface mottling typical of facial eczema in sheep and guinea pigs.

Variability of Rabbits

Although the number of rabbits so far examined is small, it is apparent that, if either the time to fall in consumption, or the amount eaten is taken as an index, there is considerable variation between rabbits in susceptibility to the toxin.

Significance of Lesions in Extrahepatic Ducts

From studies on the pathology of facial eczema in sheep and guinea pigs, McFarlane *et al.* (1958) concluded that the primary effect of the toxin is damage to the epithelial cells lining the larger bile ducts, and that all other damage is a consequence of this lesion. The route by which the toxin reaches these cells has been a matter of some discussion. In the majority of the affected rabbits, oedema of the walls of the extrahepatic ducts has been the most obvious macroscopic effect. This damage usually extends up the cystic duct to the gall bladder and in two rabbits continued into the gall bladder itself. Although the possibility that the toxin is conveyed by the systemic blood supplying these ducts, and the gall bladder, cannot on present information be entirely excluded, the appearance of such marked damage in the extrahepatic ducts is consistent with the hypothesis that the toxin is excreted into the bile, and exerts its effects after it has reached this fluid. It is certainly against an alternative hypothesis that the initial damage is to liver cells surrounding the ducts, since no such cells adjoin the external bile ducts.

Toxicity of Pasture Extracts

Rabbits X1, X2, and X3 received in their diet an ether extract of "severe" grass fractionated by the procedure described by White (1958) to the "450 concentration" stage, and Rabbit X4 received ether extract of a different sample of severe grass fractionated to the "3000" stage. The lesions in these rabbits were identical with those in rabbits fed on the unextracted grass.

Blood and Urine Examination

Blood and urine samples were collected at slaughter from eight of the affected rabbits for examination for bile pigments, but in only one was bilirubinaemia demonstrated. Three of the affected rabbits were exposed to sunlight to test for photosensitivity, but no effects were observed. Production of hepatogenous photosensitivity in rabbits is likely to be more difficult than in sheep because the level of phylloerythrin in the bile of grass-fed rabbits is much lower than in sheep or even guinea pig bile (Clare, unpublished observations).

Toxicity of Extracts of Sporidesmium bakeri *Cultures*

Subsequent to the experiments already described, Thornton and Percival (1959) produced characteristic facial-eczema lesions in guinea pigs and lambs by feeding cultures of *Sporidesmium bakeri*. An ether extract of a sporing 7-day culture of this fungus was mixed with meal and fed to two rabbits, at a level of 3.5 mg ($\equiv$ 40 ml of macerated culture) per kg body weight per day for 9 days. The mixture of extract and meal was fed each morning and when this was consumed the standard rabbit pellets were supplied. After the 9th day the rabbits continued on the normal ration. The consumption of one rabbit fell by 40% on the 11th and 12th days, but returned to the normal level subsequently, while the second animal showed no drop in intake. Both were killed on the 15th day. The livers showed lesions similar to those seen in rabbits on toxic grass.

A further portion (37 mg) of this extract was dissolved in 0.6 ml ethanol. On three consecutive days 0.2 ml aliquots were diluted with 0.2 ml of water and dosed orally to a rabbit (body weight 0.8 kg). There was no depression of intake, but when the animal was killed on the 15th day its liver showed lesions in both extrahepatic and hepatic ducts and mottled areas over most of the left lobe.

Toxicity of Sporidesmium *Spores*

Typical liver damage has also been produced in rabbits by feeding macerated cultures (mixed with meal) and by feeding spores. These spores were supplied by Dr T. R. Vernon, Plant Diseases Division, DSIR, Auckland. They were grown on an autoclaved mixture of equal weights of hay and fresh ryegrass, harvested by washing with water containing a small amount of Triton X100, freeze dried, and stored at —20°c. Concurrently with the rabbit feeding, guinea pigs were fed for the same period at levels to give a ratio spores-to-bodyweight comparable with the intake by rabbits. Results obtained for these rabbits and guinea pigs are compared in Table 2.

TABLE 2. TOXICITY OF *Sporidesmium* SPORES

Animal	Spore Sample	Spores mg/kg b.w.	Days on Spores	Days to Fall in Intake	Days to Killing	Liver Wt. (g/kg b.w.)	Liver Lesions	
							Macro.	Hist.
Rabbit A	V5	150	8	—	14	32	—	+
G.P. A	V5	230	9	—	18	35	—	? *
Rabbit B	V5	260	8	7	11	43	+	+
G.P. B	V5	360	8	—	11	38	—	—
Rabbit C	V5	230	8	11	14	37	+	+
G.P. C	V5	230	8	—	14	33	—	—
Rabbit D	V4	170	2	3	8	30	+	+
G.P. D	V4	175	2	—	8	32	—	—

* Very mild bile-duct oedema was observed.

When tested by the routine feeding procedure (21 days + 7 days on non-toxic diet) both these samples produced in guinea pigs lesions ranging from "very mild" (100 mg spores/kg body weight over 21 days) to "moderately severe" (500 mg/kg, 21 days).

In addition to demonstrating the toxicity of *Sporidesmium* spores to rabbits, these results further indicate that rabbits are more susceptible than guinea pigs to this toxic principle.

Use of Rabbit for Toxicity Assay

Although the results indicate that the white rabbit is more susceptible than the guinea pig to the facial eczema toxin, rabbits have not yet been used for routine testing of pastures or extracts, partly because comparatively few were available, but also because affected livers are less readily graded than guinea pig livers by macroscopic appearance. Considering the arbitrary nature of this grading (Perrin 1957) and the wide variation in guinea pig response to a given dose of toxin, it should be possible to devise an equally satisfactory assessment for rabbits. The time required at a fixed dose rate to produce a marked fall in consumption may provide an index to toxicity, with confirmatory liver examination. Obviously more information on the variation between rabbits, and especially on factors influencing this variation, is required.

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THE X-RAY SENSITIVITY OF *Brassica rapa* L. SEEDS

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Summary

Brassica rapa seeds were subjected to doses of X-rays from 10,000 to 100,000 r. The higher doses caused a small but significant decrease in seed germination. The survival of plants, 108 days after sowing, from seeds which had received the highest dose was approximately 15% of the survival of the control plants.

The LD 50 for *B. rapa* seeds was about 75,000 r, and the critical dose for mutation breeding purposes was about 80,000 r.

The curve relating plant survival to dose of X-rays was sigmoidal and characteristic of about 8 or 9 hits. While this might indicate a critical number of meristematic cells which have to be killed to cause embryo death, it was considered that the dose/survival relationship was complicated by lethal physiological effects of the X-irradiation.

Introduction

The sensitivity of seeds of *Brassica rapa* L. (turnip) to X-irradiation was determined as part of a project to obtain resistance to cauliflower and turnip mosaic virus diseases (Chamberlain 1954) by induced gene mutation. Sensitivity to irradiation is expressed by the dose required to kill 50% of the treated sample (LD 50), and the critical dose. The critical dose (Gustafsson 1944), is characterised by a 37% survival of mature plants, and is intended to provide a sufficiently large field material with a maximum of mutations and chromosome re-arrangements for mutation breeding purposes.

The seeds of *Brassica* species tolerate high doses of irradiation compared with the seeds of many other plants. Gustafsson (1944) found that the critical dose for *B. napus* (oil rape) seeds was above 30,000 r. and for *B. napus* var. *napobrassica* (swede) well above 90,000 r. Fujii and Matsumura (1958) measured the sensitivity of *B. rapa* var. *amplexicaulis* seeds to gamma radiation from a ^{60}Co source and found no marked differences in germination from treatments of 0 to 100,000 r, but there was a marked reduction in the number of seedlings surviving the higher doses. The LD 50, as determined from the surviving seedlings, was between 20,000 and 40,000 r in one strain, and above 100,000 r in another.

Methods

The turnip variety "Imperial Green Globe" was used. Ten packets, each containing approximately 1500 seeds, were treated with doses ranging from 10,000 r to 100,000 r at 10,000 r intervals. The irradiations were carried out with a GEC Maximar Radiotherapy Unit using 200-kVp X-rays with the tube run at 10 mA. The packets were

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irradiated in a stack of five at a time in a circular tray about 1 cm deep. The mean dose rate over the whole tray was about 33,500 (± 10%) r/hr and the maximum and minimum dose rates in the tray might have differed from the mean by about 15%. The packets were irradiated in bursts of 10,000 r (18 minutes) and in between bursts the packets were shaken and their positions in the tray changed systematically so that each packet would average as nearly as possible the mean dose rate over its total irradiation time.

Eleven days after the treatment, the seeds were sown in the field in a randomised layout of eight replicates, with two control plots in each replicate.

Results

Germination of the seeds was scored 13 days after sowing. The mean percentage germination of the seeds in each treatment, and the results of an analysis of variance for the complete data are shown in Table 1.

TABLE 1. Seed Germination 13 Days After Sowing

Dose (1,000 r)	0	0	10	20	30	40	50	60	70	80	90	100
Mean germination %	71.73	76.73	68.89	71.47	72.67	63.40	68.27	67.07	66.67	65.40	60.17	60.40

	d.f.	S.S.	m.s.	F
Between replicates	7	4,226	604	1.84
Between treatments	11	7,581	689	2.10*
Regression	1	5,545	5545	27.20**
Remainder	10	2,036	204	0.62
Error	77	25,222	328	
Total	95	37,029		

The differences between treatments and the regression of the germination response with the dose of X-rays were both significant. Increased dosage tended to cause decreased germination, although the mean germination of the seeds which received the highest dose was 81% of the mean germination of the controls. The seedlings from treatments above 70,000 r, however, were markedly smaller than the seedlings from the lower doses and from the controls. Most of the seedlings from these high-dose treatments failed to develop further.

Insect attack reduced the overall plant survival and severely damaged two of the replicates, which were rejected. The remaining six replicates were scored 108 days after sowing, at which time the surviving plants were of good vigour and could be expected to reach full maturity. The mean plant survival when related to dose of X-rays gives a sigmoid curve (Fig. 1). In an analysis of variance of the full data (Table 2), the differences between treatments and the regression of plant survival with the dose of X-rays were both significant. Further anaylsis indicated that the large variance between treatments remain-

TABLE 2. ANALYSIS OF VARIANCE OF DATA FROM PLANTS SURVIVING 108 DAYS AFTER SOWING

	d.f.	S.S.	m.s.	F
Between replicates	5	1,092	218	1.64
Between treatments	11	38,985	3,544	26.65**
Regression	1	33,198	33,198	57.34**
Remainder	10	5,787	579	4.35**
Error	55	7,298	133	
Total	71	47,375		
Analysis of Remainder:				
Curve	1	5,091	5,092	38.32**
Remainder	9	696	77	0.58
Error	55	7,298	133	

ing after the regression had been taken out could be explained by a sigmoid relationship between plant survival and X-ray dose. Such a survival curve is expected if more than a single hit on a sensitive target is required for lethality (Lea 1946). The gamma curve, in Fig. 1, was fitted to the truncated data by Dr B. I. Hayman using a standard

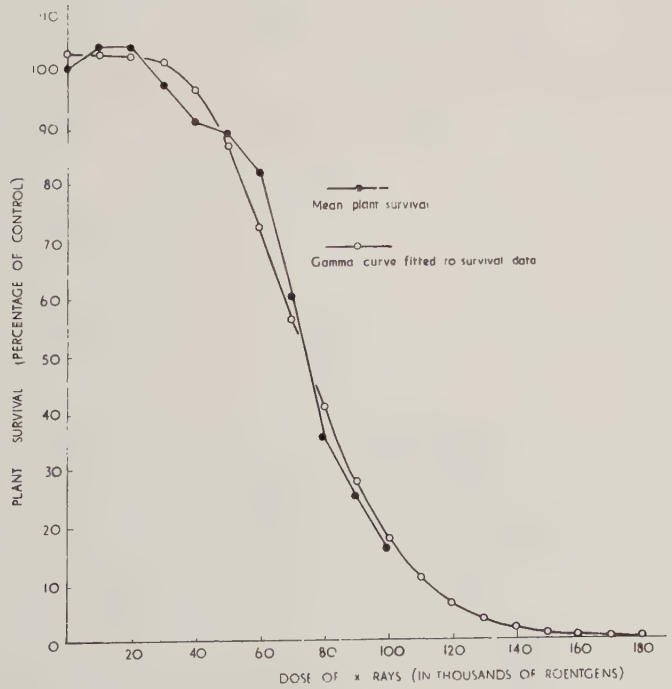

Fig. 1.—The relationship of plant survival, 108 days after sowing, to dose of X-rays.

method which permits extrapolation beyond the point of truncation. This curve is characteristic of about 8 or 9 hits.

The LD 50 for *B. rapa* seeds, as indicated by the experimental data, was about 75,000 r, and the critical dose was about 80,000 r (Fig. 1).

Discussion

The high tolerance of *B. rapa* seeds to X-rays conforms to earlier reports of the tolerance of *Brassica* species and other members of the Cruciferae.

The sigmoid relationship between plant survival and X-ray dose would be expected if lethality resulted from the cumulative destruction of sensitive elements induced by the radiation. In the case of an embryo, death may be caused by the destruction of a critical number of meristematic cells; the data would indicate about 8 or 9 cells for a *B. rapa* embryo. However, X-rays are known to produce lethal physiological effects of a more general nature and these might complicate the dose/survival relationship, so that it would not be possible to estimate a critical number of sensitive elements. MacKey (1951) obtained a sigmoid curve relating dose of X-rays to inhibition of sprouting of barley seeds, whereas with neutron treatments this relationship was exponential. The number of mutations and chromosomal aberrations produced by both radiations showed an exponential relationship to dose. To explain these effects MacKey postulated that the general pattern of low-intensity ionisations caused by X-rays induced, as well as a genetic effect, a physiological effect of high toxicity which caused the death of cells, shoots, or whole seedlings; whereas the high-density clumps of ionisation resulting from neutron irradiation did not cause physiological effects to the same extent. Caldecott *et al.* (1952, 1954) also found that, for similar chromosomal aberration and mutation frequencies, thermal neutrons produced much less seedling injury in barley than did X-rays, and they suggested that X-rays had a pronounced effect on the extra-chromosomal constituents of the cell, and probably killed the cells by non-genetic injury. In this connection it is of interest that Sax and Swanson (1941) found in *Tradescantia* that besides direct hits which produced chromosomal breaks and gene mutations, X-rays produced general physiological changes in the cell, which resulted in a cessation of mitosis, and clumping and stickiness of metaphase and anaphase chromosomes. These effects were lethal in cells that had received heavy doses.

The high doses of X-rays received by the *B. rapa* seeds could well be expected to have caused physiological effects which contributed to the sigmoidal relationship between the dose given to the seeds and the mature plant survival.

Acknowledgements

I wish to express my thanks to the Director and Dr G. S. McNaughton of the Institute of Nuclear Sciences, Department of Scientific and Industrial Research, for carrying out the X-ray treatment; to Dr B. I. Hayman for statistical advice; and to Miss Elizabeth Stevenson for the necessary computation.

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BLACK BEETLE *Heteronychus sanctae-helenae* Blanch., IN PASTURES IN NEW ZEALAND

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Summary

The black beetle, *Heteronychus sanctae-helenae* Blanch., is an important pest of pastures in New Zealand. At present the beetle is confined mainly to coastal areas, north and south of Auckland city and north of Gisborne.

Both larvae and adults are directly responsible for damage to pastures. The insect shows a preference for *Paspalum dilatatum* Poir., and is usually more abundant in soils which are either light and friable, sandy, or of a peaty nature.

In this country, outbreaks of epidemic proportions have so far been sporadic.

The beetle has only one generation per year, and overwinters predominantly in the adult form.

Dieldrin at rates as low as 4 oz per acre gives effective control of black beetle larvae in pastures.

Introduction

The black beetle, *Heteronychus sanctae-helenae* Blanch. which is now an established pest in New Zealand, is known from St. Helena; Cape Province, Rhodesia, and Transvaal in South Africa; East Africa; Abyssinia; Madagascar; and New South Wales, Australia. In New South Wales it is a major pest, attacking maize, sugar cane, and vegetable crops (McCarthy 1934; Cumpston 1940). Damage to pastures in coastal districts of New South Wales was reported by Wallace (1946). Its occurrence in New Zealand was first recorded in March 1937, from Waiheke Island (Spiller and Turbott 1944). The same paper reports a swarm of beetles doing considerable damage to foliage of vegetables at Stanley Point, North Shore, Auckland, in December 1939. Since then, it has become an established pasture pest in this country, particularly in coastal areas in the northern half of the North Island. In recent years it was realised that the black beetle had become a serious threat to the grassland economy in certain areas, and that some effort would be required to reduce its depredations. As little was known of its life history under New Zealand conditions, it was considered necessary to investigate this aspect of the problem before evolving a practical method for its control. The life-history studies were commenced in July 1957 and carried through for a full year. The data obtained from the studies were of considerable value when chemical control trials against the pest were undertaken in December 1958.

General Description

The newly emerged adult beetle is a chestnut colour, but soon changes to a glossy black. The female is about 1.4 cm long, and, in

most cases, the male is slightly smaller. The sex difference in live specimens is readily detected by the shape of the fore tarsus. In the female, the segments are elongate and narrow, in the male they are relatively short and broad. The egg is at first ovoid, white to creamy-white in colour, and about 2 mm in length. Owing to absorption of moisture during incubation, the egg swells, and becomes significantly larger and almost spherical before hatching. The larva is similar to the grass grub, having the same habit of lying in the soil in a curled up position, but attains a greater size, and is about 2.5 cm long when fully grown. The head-capsule is light brown, the body is creamy-white or greyish-white except for the hind segments which appear black where the contents of the hind-gut show through the body wall. Fully grown final-instar larvae are uniformly creamy white in colour owing to evacuation of gut content prior to pupation. The pupa is at first pale yellow, but changes to reddish-brown before the adult emerges. Prior to emergence the adult form is distinctly visible through the pupal skin.

Fig. 1.—Stages in the life history of *H. sanctae-helenae*. Top, from left: first-instar larva, second-instar larva, third-instar larva. Bottom, from left: pupa, adult beetle.

SEASONAL HISTORY

To obtain information on the seasonal cycle of the black beetle, areas that were known to be heavily infested were sampled at regular intervals over a period of 12 months. Care was taken not to dig a sample in the same place more than once, and sampling was carried out on a time basis, using 60 minutes as a minimum period for collecting.

All specimens present in the soil at each sampling were collected and preserved for later detailed laboratory examination.

The number of larval instars was determined by measuring the head-capsule width of all larvae collected. This ensured an adequate size representation for plotting the frequency distribution of these measurements, which are shown in Fig. 2, where three instars are clearly indicated.

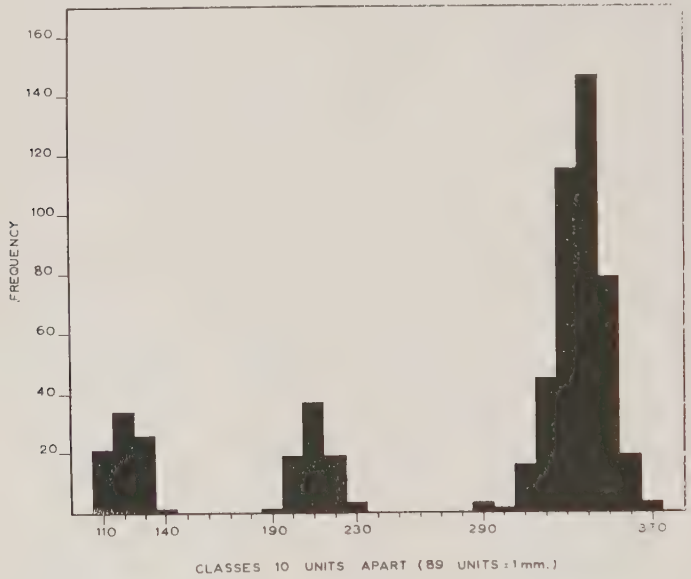

Fig. 2.— Frequency distribution of width of head capsule of larvae of *H. sanctae-helenae*.

The seasonal history studies were commenced in July 1957, and the major portion of these were carried out on a farm situated on the coast, approximately 4 miles north-east of Clevedon, but application of insecticide to the selected sampling areas during March 1958 necessitated the transference of the investigations to another area. A sample was taken from an untreated area at Clevedon in mid-April, but on account of the relatively low populations of the insect here, it was decided to abandon altogether the seasonal history studies at Clevedon. A heavily infested area at Hahei on the east coast of the Coromandel Peninsula was chosen for the continuation of the studies, and the first sample was taken from here in early May 1958. The pasture type in this area, which is situated within $\frac{1}{2}$ mile of the coast, was dominantly paspalum growing in yellow-brown friable soil. Many of the pastures in the district had been severely damaged by high black-beetle populations.

The information obtained from the 12 months' investigations showed that the main overwintering stage is the adult beetle, but owing to a certain amount of overlapping during the season it is usual

to find pupae and larvae at various developmental stages throughout this period. This is best described as a late compound brood which is non-synchronous with the main generation, and which is carried over from one season to the next resulting in the overlapping mentioned. The overwintering adults are relatively inactive, and remain in this condition in the soil from June until September. Although only observed infrequently, it is apparent that mating takes place in the soil during the spring. Harrington (1953) states that copulation takes place in the soil, and has been observed to last as long as $12\frac{1}{4}$ hours, though the actual period is probably considerably longer. Main-generation eggs are found in the soil from mid October until early January. They are deposited singly, and are usually found approximately $\frac{1}{2}$ in. below ground level, although in some cases they have been found at a greater depth. The incubation period is about six weeks early in the season, but probably decreases later when higher soil temperatures are normally experienced. The larvae pass through three instars before pupating. The new-generation beetles emerge through March, April, and May, with a small number appearing in February. By June most of these have become dormant, and remain in this condition throughout the winter. In the spring they resume feeding, and continue to feed until December, after which there is a gradual decline in numbers as they die off. It is usual to find many dead beetles on the ground at this time of the year, and very few live beetles can be found until the new

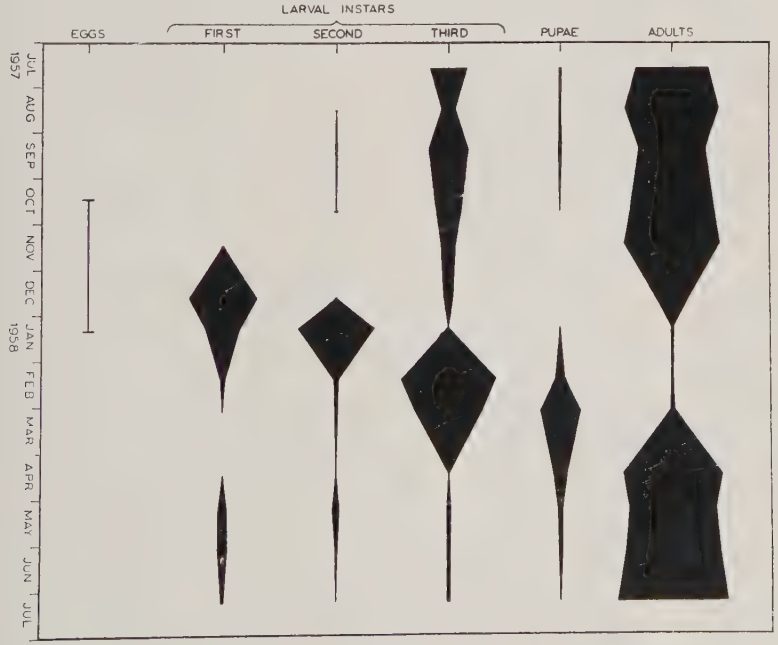

Fig. 3.—Diagrammatic representation of the development of *H. sanctae-helenae.* Widths of blocks are proportional to the percentage of the population in a particular stage excepting the egg. Main period of occurrence in eggs is indicated by a line.

generation begins to emerge. There is only one generation per year, and during the 12 months' cycle, old-generation beetles are feeding from late September until December, and new-generation beetles from March until June, a total feeding period of 6 months. The principal feeding period of the larvae is from December until February, and in some cases may even continue on into March. On account of varying climatic conditions and seasonal trends, there are probably some variations in the life cycle of the black beetle from one season to another, and from place to place. Details of the seasonal cycle studies of *H. sanctae-helenae* are shown diagrammatically in Fig. 3.

Habits

The chief breeding grounds of the black beetle in New Zealand are grassland pastures, particularly those growing in coastal areas where the soil is usually light, sandy, and extremely friable. The insect also shows a preference for peaty soils. This association with peat soils may be due to the high content of organic matter, some authors claiming that larvae use this organic matter as a food source. As large populations have not been found in either the heavier types of soil, or on hill country, it is considered most unlikely that outbreaks of epidemic proportions would occur under these conditions. Paspalum pastures are most susceptible to black-beetle invasion, and when heavy infestations occur the paspalum may disappear completely. In many cases this leaves bare ground, and a subsequent invasion of clover, flat weeds, and thistle. Most pasture grasses are open to attack. Weeds (other than *Gramineae*), and legumes, are not favoured by the insect, and are virtually exempt from attack. Wallace (1946) reports that two grasses, *Phalaris tuberosa* L., and kikuyu, are capable of withstanding very heavy beetle infestations. In New Zealand the host range of the beetle is wide and varied. Atkinson *et al.* (1956) give the following list of plants, which, in addition to pasture types, are susceptible to attack: maize and sweet corn, tomato, potato, sweet potato (kumara), carrot, cauliflower, strawberry, tobacco, marigold, and grapevine cuttings. At Hahei, adult beetles were found tunnelling into windfall fruit of apple, quince, and feijoa.

The adult beetle, which spends a large portion of its time in the soil, is voracious, and, when attacking pasture plants, feeds mainly on the root system immediately below ground level. In the case of cocksfoot, crown damage has been observed. Adult beetles do not appear to be adversely affected by wet conditions, and are able to tolerate long periods of submersion in water. During the winter, however, they do tend to favour the drier situations in which to hibernate, and in many cases are to be found beneath rushes, and under dung pats. Large numbers of live beetles have been reported floating in the sea west of Coromandel by commercial fishermen operating in this area. Their flight range in New Zealand is not known, but there is evidence to suggest that they are relatively strong fliers. Individual beetles have been seen to take off on a more or less vertical flight and disappear from view within a very short space of time. Although the author has not seen the great massed swarms of beetles as reported by Wallace (1946) in Australia, reports have been received from reliable

observers of this occurring in heavily infested areas in New Zealand, during warm autumn evenings. It would appear that these are dispersal flights; however, the possibility of their being associated with mating was examined. Female beetles dissected at monthly intervals, from April until July, were in every instance found to be unfertilised (Fig. 4A). At the beginning of October, further dissections of female beetles were made, and in this instance all specimens were fertilised (Fig. 4B). The result of the dissections gives adequate confirmation that mating does not occur during the massed flights, but in the spring, as mentioned earlier.

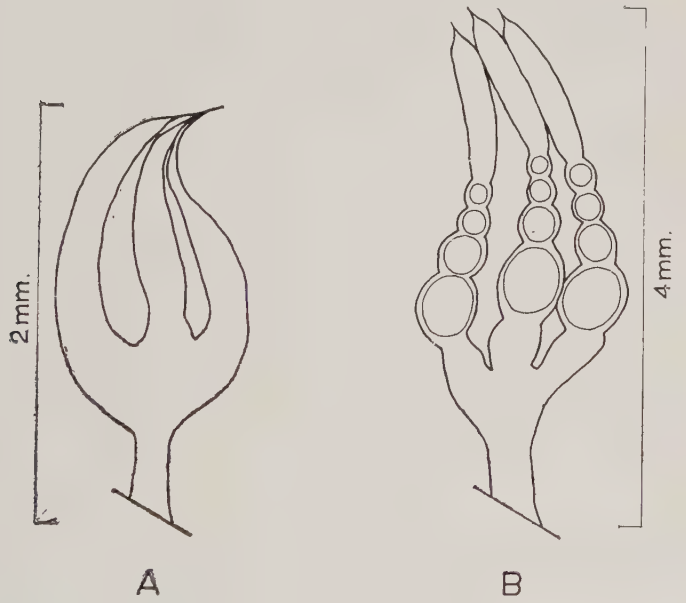

Fig. 4.—A. Three ovarioles from unfertilised beetle. B. Three ovarioles from fertilised beetle showing succession of eggs.

Harrington (1953) states that larvae of *H. sanctae-helenae* have not been observed to feed on anything but soil, which they ingest in considerable quantities, the decaying vegetable matter present in the soil being apparently of sufficient nutritional value. The ability of larvae to develop in this manner was also demonstrated in an experiment at Clevedon when first-instar larvae were placed in earthenware pots which contained soil only. Although only a small proportion of the specimens reached the pupal stage, the result was conclusive. In New Zealand it has been found that larvae of the black beetle are also capable of damaging the root system of pasture plants. This was demonstrated at Clevedon when an experiment was carried out to assess the susceptibility and nature of damage sustained by some of the more commonly grown grass species when exposed to attack by the larvae. First-instar larvae were enclosed in earthenware pots together with well established single plants of perennial ryegrass, cocksfoot, and paspalum. In each test, 6 larvae were used. The soil used was light in structure,

and did not contain a great deal of organic matter. After 5 weeks exposure the ryegrass plants had succumbed, the larvae having completely chewed through the plants just below ground level. Paspalum and cocksfoot plants were less severely affected, but in each case a significant loss in vigour had occurred from root damage. Cumpston (1940) reports that, in Australia, larvae of black beetle have extensively damaged the root system of turf. Harrington (1953) suggests that this may be due to a very poor content of decaying vegetbale matter or humus in the soil. Many of the heavily infested areas in New Zealand occur near the coast in soils of low organic content. This may explain the extensive pasture damage caused by larvae in this country. Even in areas where the soil is relatively rich in humus, and where heavy infestations have occurred, it has been possible to roll the turf back with relative ease to expose the larvae which were obviously feeding on the root system of the pasture species (Fig. 5).

Larvae are not able to endure long periods of submersion in water, and in areas subject to flooding many larvae are drowned. Under these conditions, and to a lesser degree in areas where flooding does not occur, there is a markedly irregular density distribution of larval populations. In peat soil, which usually has a fairly high moisture content, larvae tend to be concentrated in the higher situations where soil conditions are drier. On undulating country which is not subject to flooding they are often found more abundantly on slopes, particularly on those with a northerly aspect. In pastures which are more uniform in vegetation, topography, and moisture content, the larval population is more evenly distributed. It would appear that the irregular distribution of larval populations in a pasture could be determined to some degree by the preference of the female beetle for soil of a particular moisture

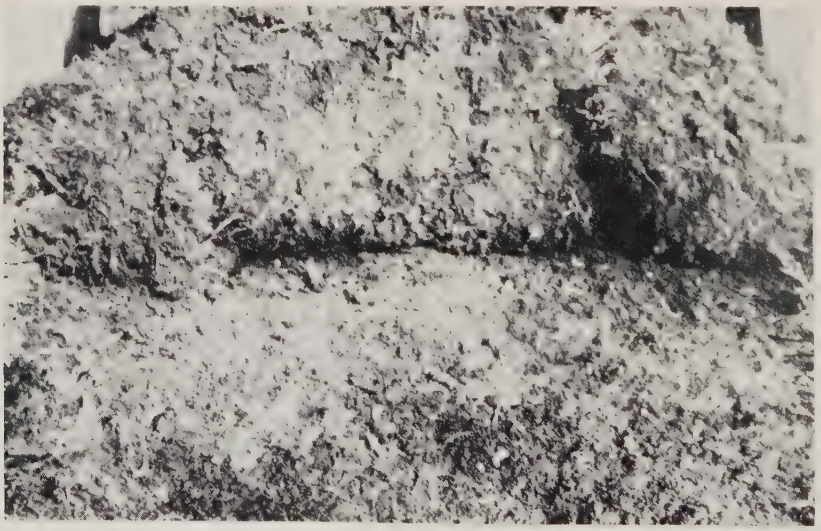

Fig. 5.—Paspalum in peat country damaged by black beetle larvae. Turf rolled back to show loss of root system.

Photo: H. McM. Bull.

content when ovipositing. Conversely, it could be associated with the autumn dispersal flights when it is possible for a large number of adult beetles to invade, by chance, specific areas in a pasture. From observations made in the field it is apparent that a form of milky disease is responsible for a substantial level of mortality in overwintering larvae and pupae. Mortality appears to be much higher where persistently wet soil conditions prevail, and it seems certain that this disease plays an important part in the natural control of black beetle in the field. Smears from larvae which had the appearance of this milky disease were examined for bacterial spores with negative results. Beard (1956) has described a milky-disease bacterium, *Bacillus euloomarahae* Beard, from Australia affecting *H. sanctae-helenae*.

DISTRIBUTION AND SPREAD

A total of 108 sampling sites in the northern half of the North Island have been examined for the presence of the insect. In most cases a minimum of 6 samples, each approximately ⅓ square foot, was taken per site, and where possible the most favourable sites were selected for sampling. The location of the sampling sites is shown in Fig. 6 where the presence of the insect is indicated by a solid black circle, and its absence by a cross. The location of earlier-collected specimens is also shown.

Since its arrival in New Zealand prior to 1937, the black beetle has spread both north and south of its initial point of establishment with sporadic outbreaks of epidemic proportions occurring in coastal regions. The most northern record on the west coast is Dargaville, and Mangawai on the east coast. The southern limit of continuous distribution of the insect appears to be a line which runs roughly between Waiuku on the west coast and Tairua on the east coast of the Coromandel Peninsula. In March 1955, adult beetles were reported in a bowling green in Gisborne. Another report from Gisborne about the same time made reference to swarms of adults during the evening. Although there has not yet been an opportunity to conduct a survey in this area, current information indicates that the insect has spread a considerable distance up the coast from its initial point of establishment in Gisborne. In February 1959 in the course of a survey of insects associated with fodder crops, a single adult specimen was taken at a light on the coast between Opotiki and Cape Runaway by Mr A. C. Eyles. The general pattern of distribution and spread of the black beetle appears to be related to pasture type, soil type and texture, and topography of the country. Generally, in areas where the population density of the beetle is high, paspalum is the dominant pasture type, and the soil is either light and friable, sandy, or peaty. It seems feasible that the natural overland spread of the black beetle could be retarded somewhat by heavily forested and mountainous country. Ocean currents and tides are no doubt important factors in promoting the spread of the pest, and probably account for the sporadic and isolated outbreaks which have occurred in coastal regions at various times. Reliable observers have on occasion reported large numbers of live adult beetles being carried out to sea by streams and open drains, particularly after flood waters have covered their breeding grounds.

Once in the sea it is possible for currents and tides to carry the beetles to other points along the coast, where, if conditions are favourable for breeding, the pest will become established. Rapid transport of sheep from one part of the country to another may possibly be another method of dispersal. It is not uncommon for live beetles to become caught in the wool of unshorn sheep, and it is therefore likely that the insect has become established in some areas in this manner.

Chemical Control

Pot Experiment

In January 1958 at Clevedon, a small-scale experiment was undertaken to test a group of insecticides against the larval stages of the beetle. The experiment was conducted in 8-in. earthenware pots in which single plants of paspalum were growing. The surface area of the soil in the pots was used to calculate the equivalent per-acre dosages of insecticides. There were 6 replicates of each treatment, the treatments being as follows:

Material	Dosage per acre (lb active ingredient)
DDT 50% wettable powder	2
Lindane 16% wettable powder	1
Dieldrin 50% wettable powder	1
Aldrin 40% wettable powder	1
Control	No treatment

The insecticide was incorporated in the soil by suspending the necessary quantity of insecticide in 600 ml water, and then drawing off and applying 100 ml to each replicate. Six first- and second-instar larvae were enclosed in each pot, and approximately one month later the numbers of surviving larvae were counted. In the control pots 47% larval mortality had occurred, in DDT pots 50%, lindane 86%, and in both the dieldrin- and aldrin-treated pots 100% mortality was recorded. At a later date the same number of third-instar larvae were enclosed in the dieldrin- and aldrin-treated pots. Four untreated pots were used as controls. When examined after three weeks' exposure, the mortality in the control pots was 29%, dieldrin 86%, and aldrin 81%. The results of the experiment showed that DDT was ineffective, lindane partially effective, and dieldrin and aldrin both highly effective.

Field Experiment

In December 1958, at Pollock, a replicated field trial was laid down against the larval stages of the black beetle employing a dieldrin-superphosphate dry mix at dosages of 4, 8, and 16 oz of active ingredient per acre, and a commercially produced dieldrin-superphosphate wet mix at 8 oz active ingredient per acre. In each case the superphosphate was applied at the rate of 2 cwt per acre. The dry-mix material was mixed in a concrete mixer for 3 minutes with the bowl revolving at approximately 25 rev/min. The experimental area was a paspalum-dominant pasture with clover, growing in brown friable soil on the south-west coast of Manukau Harbour. Preliminary counts made of the black-beetle population in the area in November showed that there was an average of 12 per square foot (with a coefficient of

Fig. 6.—Known distribution of *H. sanctae-helenae* in New Zealand 1959. A solid black circle indicates presence of insect. A cross indicates absence of insect.

variation of 32%) consisting mainly of eggs and previous season's adults. The experiment was laid out as a randomised block with four replications of each treatment. The individual plots were $\frac{1}{2}$ chain square ($\frac{1}{40}$ acre), with buffer areas two feet wide between plots. The treatments were applied by hand on 20 December, and at the time of application conditions were calm, and the pasture was relatively dry and short. Steady rain fell at the conclusion of the treatments. The plots were sampled on 16 February, 58 days after treatment. Eight samples, each 7 × 7 × 5 inches, were taken at random within each plot, and the number of living black beetle larvae were recorded for each sample. The counts of surviving larvae per treatment, and their standard errors, are presented in Table 1.

Statistical analysis of the data relating to surviving larvae, reveals that all treatments gave a significant improvement over control, and that all the dry-mix treatments were significantly more effective than the wet-mix. Within treatments other than wet-mix, there was a significant increase in effectiveness with increase in rates of application, except that the difference between 4 oz and 8 oz did not reach significance.

TABLE 1. Counts of Surviving *H. sanctae-helenae* Larvae 58 Days after Treatment

Treatment	Dosage per Acre (oz active ingredient)	Total Number Living Larvae per Treatment and their S.E.
Dieldrin (dry mix)	4	16 ± 3.9
Dieldrin (dry mix)	8	6 ± 3.6
Dieldrin (dry mix)	16	0 —
Dieldrin (wet mix)	8	42 ± 4.9
Control	No treatment	77 ± 5.8

Analysis of variance was performed on transformed data, but true totals and decoded S.E.s are given above.

Discussion

A study of the ecology of black beetle, *Heteronychus sanctae-helenae* in pastures in New Zealand has enabled the status of this insect to be viewed with greater clarity than has been possible in the past. From the results of the experimental work carried out on the insect, it has been possible to evolve a satisfactory method for controlling it in the field.

Seasonal history studies show that there is only one generation of the beetle per year. Natural dispersion of the insect occurs in the autumn, and mating takes place in the soil during the spring.

Both larvae and adults of the black beetle are directly responsible for damage in pastures, so that pasture deterioration is continuous.

The insect favours paspalum as a host plant, and is usually more abundant in soils which are either light and friable, sandy, or peaty. Occurrences of the beetle in epidemic proportions are sporadic and confined mainly to coastal areas. Such occurrences result in serious economic loss caused by lower stock-carrying capacity, and, in some cases, forcing the sale of stock. Often there is the added cost of cultivation and re-grassing. Continued pressure of low populations of black beetle in pasture in the absence of visual damage, could cause a progressive deterioration in plant composition, and a subsequent drop in pasture production.

Information obtained from the distributional studies of the black beetle, suggests that it has not yet invaded all the country suitable for its survival, and that it will continue to extend its range. It is probable that black beetle occurs unrecorded in other areas, as yet, at low population densities.

The results of the insecticidal trial demonstrated that satisfactory control of *H. sanctae-helenae* larvae in pastures can be secured with dieldrin at dosages as low as 4 oz of active ingredient per acre. For practical purposes however, and to compensate for faulty application of

the material, it is considered that 8 oz of active ingredient per acre should be used.

Kelsey (Anon. 1959) commenting upon the development of wet-mix DDT-superphosphate for control of grass grub, *Costelytra zealandica* (White) in pastures, said: "It is slower acting than the dry-mix, but we have had effective results from the wet-mix, even on heavily infested areas." The trial at Pollock showed that the commercialy produced wet-mix dieldrin-superphosphate was slower acting than the dry-mix material, and that consideration of this factor would be necessary when control of black beetle is contemplated. The above statement is based on the assumption that the correct quantity of dieldrin was included in the commercially prepared product.

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